6th Hiroshima Conference
on Education and Science in Dentistry

1965 - 2015
50th Anniversary Commemoration

BioDental Education and Research Towards the Next 50 Years

Hiroshima University Faculty of Dentistry

ISSN 0046-7472

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Proceedings of 6th Hiroshima Conference on Education and Science in Dentistry
October 23-25, 2015, in Hiroshima, Japan

Hiroshima University Faculty of Dentistry

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Printed in Japan

PREFACE

On behalf of organizing committee members of Hiroshima Conference and Hiroshima University Faculty of Dentistry, it is my great pleasure of extending to you an invitation to participate in 6th Hiroshima Conference on Education and Science in Dentistry with the theme, "BioDental Education and Research towards the Next 50 years - 50th Anniversary Commemoration" to be held in Hiroshima, Japan on October 23-25, 2015.

We held the 1st Hiroshima Conference on Education and Science in Dentistry to commemorate the 40th anniversary of the founding of the Faculty of Dentistry in January 2006. Time flies so fast and I am now filled with deep emotion when I look back the previous 10 years that have passed since the 1st Hiroshima Conference. Ten years ago, coincidentally, Dental technician school and Hygienist school (2-year college) were reorganized as one school and it started as School of Oral Health Sciences as 4-year university, which is doubling the joy of this commemorable occasion.

One of the important characteristics and uniqueness of this conference is its organization: you may notice education sessions and science sessions together in this as well as previous Hiroshima Conference. This idea was originated from the belief of the founder of Hiroshima Conference, Prof. Kurihara, "the advanced research is indispensable to the advanced education". Within these 10 years, our school has significantly grown and transformed under the key concept, BioDental education and research.

I strongly wish all of you to enjoy state of the art special lectures on science and education, presentations of young investigators from various countries, stimulate discussions, develop international and inter-school collaborations, and think together what we should do in dental education and research towards next 50 years! And please plan to join Hiroshima University Faculty of Dentistry 50th Anniversary Cerebration at the room Sunflower on Oct 24 afternoon with our distinguished guests, international alumni and friends.

All the best,

Motoyuki Sugai, DDS, PhD

President, 6th Hiroshima Conference on Education and Science in Dentistry

Dean, Faculty of Dentistry Hiroshima University

Congratulatory Address on the 50th Anniversary of the HU Faculty of Dentistry

Today, on the occasion of the 50-year anniversary of the Hiroshima University Faculty of Dentistry, I would like to say a few words of congratulations.

The origins of Hiroshima University's Faculty of Dentistry can be traced back to April 1st, 1965, when it was established as the third national university's dental school after Tokyo Medical and Dental University and Osaka University's Faculty of Dentistry. The establishment of the Faculty met the demands of a broad range of Hiroshima Prefecture's population, and it was the Hiroshima Prefecture Dental Association which first proposed that the Faculty of Dentistry become an official part of the university.

In 2005, the School of Oral Health Science was founded, and since its inception it has greatly contributed to the creation of excellent dentists, dental technicians and dental hygienists, active throughout Japan.

The 21st century is an international era of low birth rates and longevity, and globalism extends to every part of the world. Also in the field of dentistry, global-standard knowledge and technical skills are required.

The Hiroshima University Faculty of Dentistry provides high level dental medicine, medical services and oral health science, based on life sciences. It also fosters dental specialists, able to be active in an aging society and globalized environment.

Additionally, in 2012 an "International Dental Course" was established, resulting in Hiroshima University accepting international students from all over Asia, and becoming a nationwide pioneer in teaching lectures both in English and Japanese. Thus, via this multicultural educational environment, our university is also making efforts to foster the future leaders of dental medicine.

In 2013, Hiroshima University was elected to be part of the "Program for Promoting the Enhancement of Research Universities", together with 21 other institutions, and in 2014, our university became one of 13 members to take part in the "Top Global University Project" as a "Type A (Top type)" university.

Participating in these two projects means that Hiroshima University has the potential to "be ranked in the world's top 100 universities within the next ten years", which comprises one of the milestones along the road towards our long-term goal.

Looking ahead, Hiroshima University aims to become a "university with sustained world-wide fame and splendor even after 100 years", by continuously fostering "peace-pursuing, cultured personnel with international experience", amidst close cooperation with the Faculty of Dentistry, the Graduate School of Biomedical & Health Sciences and other departments.

I expect that the Faculty of Dentistry will continue to dedicate itself to producing excellent dental professionals who are active in global and local communities, always considering the perspective of their patients.

In conclusion, I would like to ask all of you here today for your continued understanding and support, and I would also like to offer a prayer for the continuing growth and prosperity of the Faculty.

Sincerely,

Mitsuo Ochi

President, Hiroshima University

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Dental Research and Training in the 21st Century: A Perspective from the National Institute of Dental and Craniofacial Research, NIH

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ABSTRACT

The Sixth Hiroshima Conference on Education and Science in Dentistry provides an opportunity to describe the research and training efforts of the National Institute of Dental and Craniofacial Research, National Institutes of Health, USA, which will guide our research endeavors as we look forward to the next 50 years of dental science. As highlighted below, major advances in research tools and technologies have resulted in marked insights into drivers modulating health and disease and some of these discoveries are transforming the quality of health of all communities. Selected topics covered include: a) NIDCR investments in tools and technologies; b) examples of basic research that have led to products; and c) NIDCR networks and consortia.

INTRODUCTION

The mission of the National Institutes of Health (NIH) is to seek fundamental knowledge about the nature and behavior of living systems and to apply that knowledge to enhance health, lengthen life and reduce illness and disability. The NIH is the nation's medical research agency and the world's largest source of funding for biomedical research. The NIH is made up of 27 Institutes, Centers and Offices each supporting a broad array of basic, translational, clinical and social and behavioral research. The NIH also funds research training to help grow and strengthen our national research capacity. Most of the NIH budget supports academic research laboratories, private research institutions and small businesses as well as international research organizations. Approximately 10% of the NIH budget supports an intramural research program located mostly on our campus in Bethesda, Maryland. This intramural funding includes support for the NIH Clinical Center, the world's largest hospital dedicated to clinical research. Most NIH research support goes to projects that are investigator initiated, reviewed by peers for their meritorious science, and funded by the various Institutes based on their research priorities. A portion of the NIH budget supports programs that are of high risk or are cross-cutting throughout the NIH involving topics of interest to most Institutes and Centers. Such initiatives currently include projects focused on the human microbiome, epigenetics, glycomics, undiagnosed diseases, health care systems research collaboratory, and big data.

The National Institute of Dental and Craniofacial Research (NIDCR), the third Institute of the NIH, was established in 1948 due to the growing caries epidemic and the consequences i.e. loss of almost all ones teeth by age 21 and a need to understand the disease and overcome the consequences. Since that time the NIDCR has expanded its portfolio substantially, supporting the majority of dental, oral and craniofacial (DOC) research and almost all of the DOC research training at NIH (Figure 1). Our mission is to improve dental, oral, and craniofacial health through our support of research, research training, and dissemination of health information. We want to be recognized as a catalyst of change in transforming how oral health care is delivered to our communities. The NIDCR funds a broad portfolio of research across the nation that is mainly investigator-initiated but also that is driven by emerging, new opportunities and identified areas of underrepresented support. Some current examples or priority research for NIDCR include: precision medicine, our Dental Practice-based Research Network, the oral microbiome, tissue engineering/regenerative medicine, dental health disparities, E-cigarette aerosols, and pharmacogenomics of orofacial pain. NIDCR also supports an extensive training and

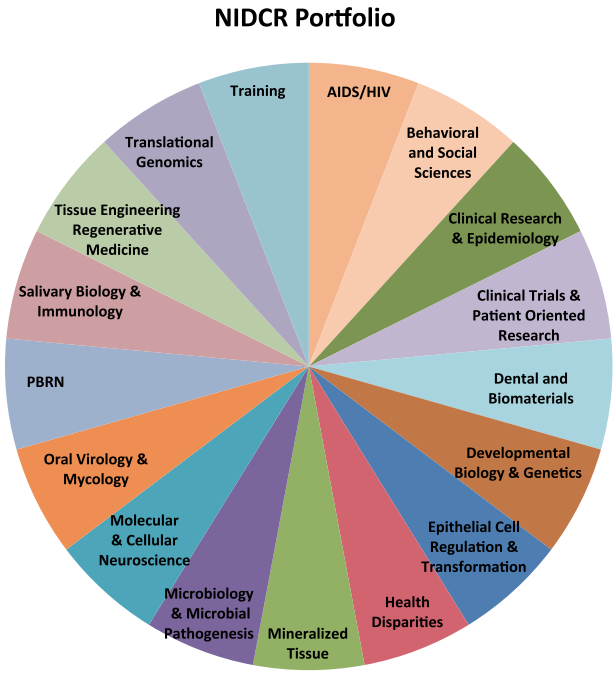

Figure 1. Pie chart reflects the diversity of NIDCR-funded research. (Please note: the pie fractions are not related to distribution of funding.)

career development program ranging from curious young high school students to established senior investigators seeking advanced training in new methods of contemporary biomedical science. In addition, NIDCR provides formal training in a dental public health residency program focused on oral and craniofacial health-related epidemiologic research. Through these mechanisms, NIDCR strives to establish a trained and diverse workforce of scientists and clinicians at the forefront of research to advance oral health care and reduce health disparities.

NIDCR Research: Advancing Tools and Technologies

NIDCR encourages the development and application of new tools and technologies to advance scientific knowledge and improve oral health. Rapid advancements in technologies have enabled the collection of large data sets including genome-wide sequencing, proteomics, epigenetics, microbiome analyses, and patient cohort data. However, to be most useful, this information requires considerable resources to store, analyze, and disseminate in a manner that allows for effective cross-disciplinary use. Towards this goal, NIH has instituted a data sharing policy requiring that all genomic data are shared, established an NIH Office of Data Science, and launched a trans-NIH Big Data to Knowledge (BD2K) initiative. The BD2K initiative aims to facilitate broad use of digital biomedical data, improve the tools needed to analyze biomedical big data, support centers and systems for big data discovery and collection, and enhance training in biomedical big data science. Thus, the resources provided by BD2K should enhance access to and use of a range of big data collected in NIDCR's research portfolio, including a number of genome-wide association studies (GWAS).

NIDCR supports GWAS and next-generation sequencing efforts to better understand the underlying genetic factors contributing to DOC diseases and disorders including oral cancer, temporomandibular joint disorder (TMD), craniofacial development and disorders, caries and periodontal disease, and Sjögren's syndrome (SS). For example, NIDCR-supported scientists recently reported on the effects of variations in enamel matrix genes and fluoride exposure on dental caries[1]. Their data indicated that the effects of two identified genetic variants are modulated by fluoride exposure, as participants with the risk genotype only exhibited greater incidence of dental caries if they were exposed to less than 0.7 ppm fluoride in drinking water[1]. Additionally, genetics can greatly influence an individual's responses to drugs and other therapeutic agents. As such, pharmacogenomics will enable a better understanding of the genetic variations that may influence drug metabolism, therapeutic response, and side effects. To encourage more research in this area, NIDCR has proposed a new initiative for pharmacogenomics of orofacial pain management. Additionally, by combining genetic, molecular, and 3D imaging technologies, the NIDCR-funded FaceBase consortium and our intramural clinical center staff are collecting genomic and gene expression data along with human facial imagery to help identify the genetic changes underlying facial development[2]. These resources provide a unique opportunity for craniofacial researchers to better understand craniofacial development and disorders. Overall, NIDCR has a broad portfolio of research to investigate how genetic and epigenetic variations influence health and disease and to evaluate the interplay between environmental factors and genetics (see Table 1 for a list of various Data Sharing resources and corresponding websites).

Table 1. Data Sharing Resources

Database Sharing Resource	Web address
NHANES NIDCR and NIH partner with other Federal agencies	http://www.cdc.gov/nchs/nhanes.htm
The Cancer Genome Atlas (TCGA) and International Cancer Genomics Consortium	http://cancergenome.nih.gov/ https://icgc.org/
Global Alliance for Chronic Diseases	http://www.gacd.org/
Human Microbiome Project and the Human Oral Microbiome Database	http://hmpdacc.org/ http://www.homd.org/
The Sjögren's Syndrome International Collaborative Clinical Alliance (SICCA)	https://sicca-online.ucsf.edu/
MD Anderson Salivary Gland biorepository	https://research.mdacc.tmc.edu/Salivary_DB/
Oral Cancer genome database	http://www.tumor-gene.org/Oral/oral.html
FaceBase	https://www.facebase.org/
Salivary proteome wiki	http://salivaryproteome.nidcr.nih.gov/
NIH's database of Genotypes and Phenotypes (dbGaP)	http://www.ncbi.nlm.nih.gov/gap
Big Data 2 Knowledge (BD2K)	https://datascience.nih.gov/bd2k
The National Dental Practice-Based Research Network (NDPBRN)	http://www.nationaldentalpbrn.org/
GTEx consortium	http://www.gtexportal.org/home/
Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPERA)	https://www.oppera2.org/OPPERAII/FAQs

In addition to better understanding genetic factors underlying disease, NIDCR invests in research to identify biomarkers and to develop salivary diagnostics for early disease detection and prevention. One recent example of this includes an NIDCR-supported study to detect tumor DNA in saliva, using somatic mutations and HPV genes as tumor DNA biomarkers[3]. Tumor DNA detection in saliva was 100% effective for oral cancer detection and 47-70% effective for detecting other cancers tested. Moreover, tumor DNA could be detected in patients with cancer recurrence, but not in patients without disease recurrence[3]; therefore, tumor DNA detection in saliva may present an effective and non-invasive screening strategy for cancer recurrence following chemotherapy and/or surgery. In addition to salivary diagnostic applications in the DOC region, studies are also underway to determine if saliva may be effective for the detection of autism spectrum disorder and for identifying biomarkers predictive for maladaptive stress reactions such as post-traumatic stress disorder. Therefore, salivary diagnostics have the potential for broad cross-disciplinary applications[4,5]. The oral cavity also presents an ideal location to use biosensors for monitoring health and disease. While small wireless biosensors are still in early stages of development, they have the potential to provide valuable dynamic, real-time diagnostic and physiologic information; thus, oral biosensors are an area of interest for current and future NIDCR research.

NIDCR Research: Basic Research to Product Development

Basic science research funded by NIDCR has not only enhanced knowledge in the field, but also led to applied improvements in DOC health with the development of novel and improved treatments for a variety of diseases and conditions. One such innovation developed by NIDCR researchers is alkaline phosphatase replacement therapy for skeletal and dental defects related to hypophosphatasia (HPP). HPP is a disease of brittle bones and tooth loss that is caused by deficiency in the enzyme, alkaline phosphatase[6]. Basic research on this enzyme was initiated in the 1990s and continues to be funded by NIDCR, and the translation to product has been through public-private partnerships. Clinical trials for enzyme therapy to treat skeletal dysplasia were completed in 2010, with very positive outcomes[7], and this treatment was granted Breakthrough Therapy designation by the Food and Drug Administration (FDA) in 2013 to expedite the approval and development process.

Another example of basic research leading to innovative treatment involves the application of the fibromodulin peptide to reduce scarring and correct birth defects including cleft lip. The role of fibromodulin in promoting scarless wound repair was discovered while investigating the transition from fetal wound repair, which is scarless, to wound healing in adults, which results in scars and fibrosis, in rodent models. Researchers discovered that fibromdulin was induced in fetal wound repair but not after the transition to adult-type repair, indicating this molecule could be important for scarless wound healing[8]. Starting with an exploratory basic science research grant, development of a therapeutic peptide derived from native fibromodulin[9] into a product for scar treatment has progressed through two phases of NIDCR-funded small business grants and is anticipated to enter into a clinical trial in 2016.

Research from NIDCR's intramural program led to the proposed use of aquaporin-1 (AQP1) gene therapy for treatment of dry mouth induced by head and neck cancer radiation treatment[10,11]. Approximately 85% of head and neck cancer patients undergoing radiation treatment experience complications from dry mouth including dental caries, enamel erosion, oral infections, weight loss and malnutrition, and difficulty talking, chewing, swallowing. While many will recover some gland activity over time, at least half will not, resulting in a long term chronic condition with no conventional therapy. Aquaporins are key proteins important for saliva production and salivary gland function[10,11]; thus, AQP1 gene therapy is being tested to improve saliva production to mitigate the effects of dry mouth and has completed a Phase I clinical trial. In this trial about half the patients experienced a reduction in symptoms related to the radiation induced xerostomia. These responses were dependent on the dose of the vector used to deliver the AQP1 gene, with participants who did not respond having vector related inflammation. Currently a second trial is being planned using a new vector which was shown to be less immunogenic and can result in long term activity in preclinical studies.

Another product currently in clinical trials that was initiated from NIDCR research funding is ClinRinse, a mouthwash for gingivitis. The effective molecule in ClinRinse is a lipoxin-like drug, which derives from a class of lipids that were shown to be potent modulators of inflammation and associated pain and tissue destruction[12-15]. Beyond applications to gingivitis, lipoxin could have therapeutic benefits for a variety of other inflammatory diseases to reduce tissue damage and bone loss and resolve inflammation. Lipoxin also provides a valuable alternative to the use of antibiotics. Limiting the use of antibiotics and identifying therapeutic alternatives will reduce the burden of antibiotic resistance the medical field is currently facing. An additional example of this involves NIDCR intramural researchers, collaborating with extramural researchers, to discover anti-IL-17/IL-21 treatment as a potential effective therapy for leukocyte adhesion deficiency 1 (LAD-1)[16,17]. LAD-1 is a rare genetic immune system disorder that causes severe periodontitis and other systemic inflammatory reactions. LAD-1 patients frequently receive broad spectrum antibiotics for life and are treated with steroids and antibiotics during inflammatory reactions, with limited success; more directed treatments could improve the healthcare and quality of life for these patients.

NIDCR: Developing Networks and Consortia

Research progress will come at the interface of different disciplines and through networks and collaborations to collect, analyze, and disseminate valuable information. As such, communication between researchers and dental practitioners, as well as other health professionals, is essential to ensure directed efforts for improving DOC health. Towards this goal, NIDCR supports the National Dental Practice Based Research Networks

(NDPBRNs) to bridge the communication between clinical practice and basic research. One example of this is an HPV16-screening NDPBRN study currently underway to collect more information regarding HPV16 prevalence and risk assessment for oral cancer.

Additionally, NIDCR participates in the trans-NIH microbiome project and funds efforts to characterize the oral microbiome[18]. Another new trans-NIH research focus is on precision medicine, particularly with President Obama's new proposed Precision Medicine Initiative[19]. Much of NIDCR's research fits into precision medicine including GWAS as discussed above and the patient-focused application of treatments. NIDCR also has several cohorts that fit into the precision medicine agenda of collecting patient cohort data, including OPPERA (Orofacial Pain: Prospective Evaluation & Risk Assessment), a cohort of healthy individuals being monitored for the development of temporomandibular joint disorder and other chronic pain conditions[20]. Expansion of current cohorts and sharing of data through improved electronic health records will enable valuable information to be gained about disease risk factors and prevalence.

SUMMARY: NIDCR, The Catalyst Transforming Health Care

Importantly, NIDCR supports basic research efforts to enhance knowledge and improve DOC health; yet these discoveries, products, and innovations transcend beyond the dental and oral health communities and have applications to other areas of general health. Imagine a future where: a) prevention is possible for all communities due to an in depth understanding of the genetics, environments/epigenetics, behavioral and cultural factors contributing to DOC diseases and implementation and dissemination processes are in place; b) tools and technologies have advanced, to include salivary diagnostic and imaging systems, that more precisely diagnose diseases and can also serve as risk assessment tools; c) treatments have advanced, with more targeted therapies, and improved materials and technologies for restoring tissues of the DOC complex; and d) electronic health records are used across disciplines and nationally/internationally resulting in more precise and individualized healthcare systems. Research requires multidisciplinary efforts and collaborations across all communities. It is certain that with the new knowledge gained from our collaborative research efforts and the translation of this knowledge into practice, we will enjoy an expansion in the quality of health for all of our communities.

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Session II

Oral and Systemic Disease Connection

The Oral and Systemic Disease Connection

Forsyth Institute
T.E. Van Dyke

Dental Infection of *Porphyromonas gingivalis* Exacerbates Pathological Progression of Non-Alcoholic Steatohepatitis (NASH)

Hiroshima University
M. Miyauchi, H. Furusho, A. Nagasaki, S. Sakamoto, K. Ouhara, H. Kurihara and T. Takata

The Effect of Periodontopathogenic Bacteria, *Porphyromonas gingivalis*, against the Onset of Rheumatoid Arthritis

Hiroshima University
K. Ouhara, M. Yamakawa, S. Munenaga, T. Fujita and H. Kurihara

Periodontal Disease as a Possible Risk Factor for Alzheimer's Disease

National Center for Geriatrics and Gerontology
K. Matsushita

New Paradigm for the Link between Periodontitis and Systemic Diseases

Niigata University
K. Yamazaki

The Oral and Systemic Disease Connection

T.E. Van Dyke

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Key words: Periodontitis, Cardiovascular Disease, Diabetes, Rheumatoid Arthritis, Pregnancy, Pulmonary Infection, Kidney Disease

ABSTRACT

This introductory paper examines the relationship between periodontitis and systemic diseases that are concomitantly expressed in people with periodontitis. An overview of current understanding of the associations between periodontitis and cardiovascular disease, diabetes mellitus, pre-term low birth weight, pulmonary infections, rheumatoid arthritis and kidney disease will be presented. The potential mechanisms and supporting evidence will then be examined. Three mechanisms have been proposed, including spread of local infections to remote sites, inflammatory mechanisms and the complex interactions of both. The same mechanisms do not appear to be involved in all systemic diseases and the interactions are often disease specific. Data from animal models and human studies will be presented focusing on new evidence for the role of inflammation in the pathogenesis of periodontitis, diabetes and cardiovascular disease. The data support an interaction between periodontitis and the occurrence and pathogenesis of several systemic diseases. The strength of the evidence varies between systemic diseases, but as more data accumulates, the importance of periodontal health in overall health is becoming more apparent.

INTRODUCTION

Two or more diseases can occur simultaneously or sequentially in an individual where the course or severity of one disease can have an adverse impact on the other (s). A large body of evidence now exists showing an association of periodontitis with diabetes mellitus, cardiovascular disease, low birth-weight and premature infants, rheumatoid arthritis, pulmonary infections, and to a lesser degree chronic kidney disease (for current review, see J. Periodontology 84: 4 supplement, 2013). Three mechanisms have been suggested to play a role in non-oral manifestations of periodontitis (Thoden van Velzen, Abraham-Inpijn et al. 1984). These include dissemination of infection, bacterial toxins and immuno-inflammatory injury. In the case of periodontitis, clearly the dissemination of the etiologic bacteria or their toxins from the biofilm is possible. Likewise, since the pathogenesis of periodontitis is now known to be inflammatory (Van Dyke and Serhan 2003, Serhan, Chiang et al. 2008), immuno-inflammatory tissue damage is possible also.

However, the systemic disease associated with periodontitis in any given individual is also a variable and to assume that the mechanism in each case is the same is probably naive. In this introductory paper, each disease listed above will be considered separately. The strength of the evidence for the association with periodontitis will be considered, as will the likely mechanism.

Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) : COPD is characterized by progressive obstruction of airflow and inflammation of the airways. The link between periodontitis and COPD was first identified in epidemiologic analyses of NHANES and Veterans Administration data (Hayes, Sparrow et al. 1998, Scannapieco, Papandonatos et al. 1998, Scannapieco and Ho 2001). Smoking is the single greatest risk factor for COPD, but the association in NHANES data remains even after adjusting for smoking (Garcia, Nunn et al. 2001). No data are available relating to the mechanism of action although increased airway inflammation stemming from periodontal inflammation has been suggested. Systematic reviews by Scannapieco and Azarpazhooh (Scannapieco, Bush et al. 2003, Azarpazhooh and Leake 2006) concluded that evidence was poor supporting a weak association and more studies are needed.

Pneumonia : Pneumonia can be acquired in the community, but it is also a frequent nosocomial infection. In hospitals and nursing homes, patients often have poor oral hygiene and the oral cavity can be colonized by organisms that cause pneumonia, including periodontal organisms (Scannapieco, Stewart et al. 1992). While there is no apparent association between periodontitis and community acquired pneumonia, there is fairly strong evidence that nosocomial pneumonias result from oral organisms and that oral hygiene preventive measures have a significant impact reducing risk up to 11.7% (Sjogren, Nilsson et al. 2008).

The mechanism of action in the case of pneumonia has been clearly demonstrated. Aspiration of bacteria from the oral cavity to the lower respiratory tract is a direct infectious process. Importantly, it has also been documented that proper preventive care can have a major impact.

Chronic Kidney Disease

Kidney damage with decrease glomerular filtration rate of more than 3 months is a significant public health problem worldwide associated with aging, diabetes, hypertension, obesity and cardiovascular disease (Levey and Coresh 2012). The Atherosclerosis Risk in Communities (ARIC study found a significant association between periodontitis and chronic kidney disease (Kshirsagar, Moss et al. 2005, Kshirsagar, Offenbacher et al. 2007), when adjusting for all of the above confounders. Importantly, a prospective study of Type 2 diabetes in the Pima Indian population found that periodontal disease predicted overt end stage renal disease in a dose dependent manner (Shultis, Weil et al. 2007). Nevertheless, the complexity of chronic kidney disease pathogenesis and its association with diabetes and cardiovascular disease makes implication for a role of chronic periodontitis challenging. There are no data implicating dissemination of infection vs. disseminating inflammation that may stem from periodontitis in chronic kidney disease.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a classic inflammatory disease of unknown etiology with persistent synovial inflammation that damages articular cartilage and underlying bone (Scott, Wolfe et al. 2010). There have been reports from small studies of associations between periodontitis and RA and the similarities in the pathogenesis of both disease makes the potential association very attractive (de Pablo, Dietrich et al. 2008), especially the association with citrulinated peptides (Klareskog, Catrina et al. 2009). The common mechanism would appear to be purely inflammatory, but citrulination of peptides has been suggested to be a property of the periodontal pathogen, *Porphyromas gingivalis* (Marchant, Smith et al. 2013). More detailed discussion of the relationship between periodontitis and RA follows in later presentations.

Adverse Pregnancy Outcomes

Pregnancy is a normal, healthy physiologic process that sometimes has adverse outcomes, including low birth-weight, pre-term birth (prematurity), growth restriction, preeclampsia, pneumonia and miscarriage (still-birth). Adverse pregnancy outcomes are associated with local and systemic inflammation and intra-uterine infections. Numerous epidemiologic and animal studies support the relationship between periodontitis and adverse pregnancy outcomes; however, few prospective intervention trials have supported the association (Sanz, Kornman et al. 2013). The mechanism of action probably involves both direct infection and inflammation. Our understanding of the pathogenesis suggests that inflammation cause the adverse outcomes. However, since intra-uterine infections are involved, there is reasonable support for direct infection causing the inflammation. Most adverse outcomes originate from ascending vaginal infection or blood born infections from known or unknown non-genital sites. Maternal periodontitis is a source of microorganisms in the blood and oral organisms such as *Fusobacterium nucleatum* have been found in amniotic fluid, placenta and chorioamniotic membranes of women delivering prematurely (Han, Redline et al. 2004).

Diabetes

Both type 2 diabetes and periodontitis are complex chronic inflammatory diseases. Type 1 diabetes, an autoimmune disorder resulting in loss of beta cells and insulin, is also associated with periodontitis. Here, we will focus on type 2. It is clear from epidemiologic studies that severe periodontitis directly impacts glycemic control in diabetes as well as other diabetic complications (Chapple, Genco et al. 2013). Likewise, poor glycemic control impacts periodontal outcomes (Chapple, Genco et al. 2013).

The Role of Inflammation : In recent years, it has become clear that control of inflammation prevents and reverses periodontitis. Periodontal infections are commensal infections characterized by dysbiosis of the biofilm (Hajishengallis, Liang et al. 2011, Hajishengallis, Darveau et al. 2012). Importantly, inflammation drives the dysbiosis and pharmacological control of inflammation reverses dysbiosis (Hasturk, Kantarci et al. 2007). Lipid mediators of inflammation (eicosanoids) have long been known to provide the acute inflammatory stimulus; more recently, pathways of resolution of inflammation have also been uncovered (Table 1). Importantly, natural resolution of inflammation and pharmacological antiinflammation (inhibition, antagonism) are not the same thing. Natural mediators of resolution of inflammation have provided new pharmacologic tools to determine the role of inflam-

Table 1. Lipid mediators of inflammation.

Lipid Mediators of Inflammation
Proinflammatory
• Arachidonic acid derived:
• Prostaglandins
• Leukotrienes
Proresolution
• Arachidonic acid derived:
• Lipoxins
• Aspirin triggered Lipoxins*
• ω-3 fatty acid derived:
• E-series resolvins (EPA)
• D-series resolvins (DHA)
* The role of Aspirin: Aspirin enhances lipoxin and resolvin activity by producing longer acting isomers of native compounds

The pro- and anti-inflammatory eicosanoids derived from arachidonic acid and omega-3 fatty acids are identified as Proinflammatory and Proresolution. Proinflammatory eicosanoids derived from arachidonic acid are the products of cyclooxygenases (1 and 2) and lipoxygenases (5, 12 and 15). Proresolution eicosanoids are products of lipoxygenase: lipoxygenase interactions; for review see (Serhan, Chiang et al. 2008). Depending on the substrate, lipoxins (arachidonic acid) or resolvins (omega-3 fatty acids) are produced. Aspirin has the unique property of inhibiting COX-2 by changing its activity to a 15R-lipoxygenase. The resulting proresolution products are longer acting isomers of the lipoxins and resolvins with longer half-life and greater potency.

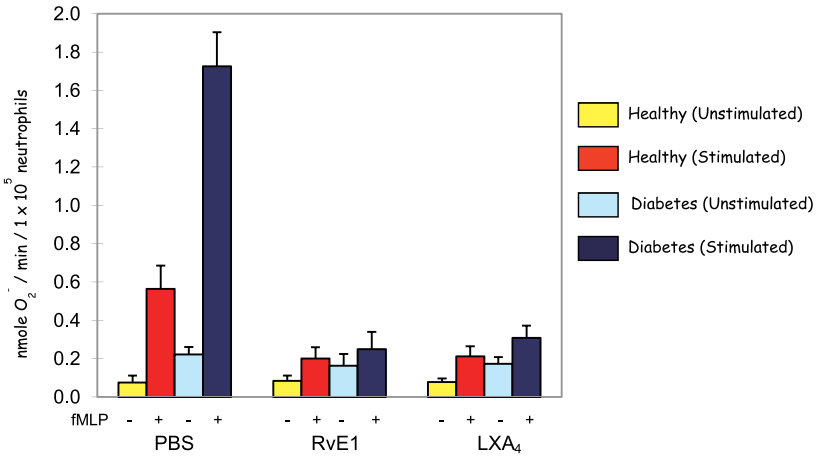

Figure 1. Neutrophil mediated oxidative stress in type 2 diabetes.
Neutrophils were isolated from peripheral blood of human volunteers with and without type 2 diabetes and stimulated with the bacterial chemotactice peptide fMLP. Unstimulated neutrophils served as control. Superoxide generation was measured using the superoxide dismutase inhibitable cytochrome c reduction assay. Additions included PBS (control) and 10.0 nM RvE1 or 10.0 nM lipoxin A_4 (LXA_4). Superoxide generation was significantly higher by diabetic neutrophils at rest and when stimulated with fMLP. Both RvE1 and LXA_4 atenuate excessive superoxide generation by neutrophils under all conditions.

mation in the pathogenesis of disease and in the context of this paper, the relationship of inflammation to associations between periodontitis and systemic diseases.

Obesity and Metabolic Syndrome : Obesity (body mass index >30 kg/m^2) and metabolic syndrome (a clustering of interrelated atherosclerotic risk factors, including abdominal obesity, dyslipidemia, hyperglycemia and hypertension) are known proinflammatory modifiers. Obesity and metabolic syndrome are both associated with periodontitis (Grundy 2005, Chaffee and Weston 2010). These conditions can eventually lead to type 2 diabetes by increasing inflammation and insulin resistance.

Inflammation Links Periodontitis and Type 2 Diabetes : Type 2 diabetes is preceded by systemic inflammation that leads to insulin resistance, reduced β cell function in the pancreas with eventual apoptosis of β cells and lack of insulin production (Chapple, Genco et al. 2013, Chapple, Borgnakke et al. 2014). Acute phase and oxidative stress biomarkers further support the role of inflammation. Inflammation can be induced by oral bacteria that get access to the circulation (Genco, Grossi et al. 2005). Sustained elevations of blood glucose levels leads to non-enzymatic glycation of proteins called advanced glycation endproducts (AGE) (Schmidt, Weidman et al. 1996) that bind to their receptor (RAGE) on inflammatory cells to increase inflammation (Lalla, Lamster et al. 2000, Lalla, Lamster et al. 2000, Lalla, Lamster et al. 2001). Thus, there are both direct and indirect proinflammatory stimuli.

Oxidative stress (reactive oxygen species [ROS], superoxide [O_2^-]) is an indicator of oxidative stress and it is greatly increased in type 2 diabetes (Figure 1). Using the resolution of inflammation agonists RvE1 and LXA_4 as molecular probes to control inflammation, we demonstrated that ROS levels could be brought back to normal in type 2 diabetes (Figure 1). Further, in type 2 diabetic mice, control of inflammation with resolvin prevented periodontitis and improved glycemic control (Figure 2), further demonstrating the role of inflammation in the

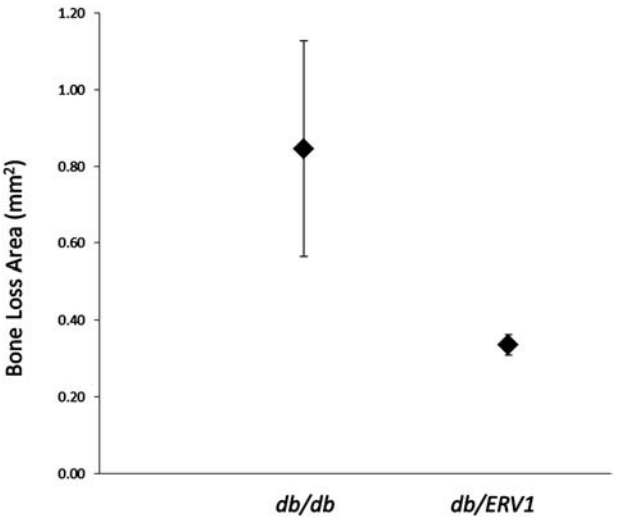

Figure 2. Control of inflammation reduces periodontitis in type 2 diabetes.
Periodontitis was induced in 2 strains of mice using *Porphyromonas gingivalis* gavage following the Baker protocol (Baker, Dixon et al. 2000). The two strains were $db^{-/-}/db^{-/-}$ (leptin knock out) type 2 diabetic mice (*db/db*) and type 2 diabetic overexpressing ERV1 (*db/ERV1*) the receptor for RvE1. The *ERV1* mouse is known to have a lowered inflammatory phenotype (Gao, Faibish et al. 2013). Type 2 diabetic mice are clearly more susceptible to periodontal bone loss. Dampening of inflammation in the *db/ERV1* mouse clearly protects the diabetic mouse from periodontal bone loss.

Periodontitis/type 2 diabetes connection.

Cardiovascular Disease

Periodontitis is a risk factor for systemic inflammatory diseases, including atherosclerosis, myocardial infarction, and stroke (Dietrich, Sharma et al. 2013). Recent systematic reviews suggest a significant risk association. Clinical studies also demonstrate that people with periodontitis have elevated C-reactive protein (CRP), interleukin 6, haptoglobin, and fibrinogen. People who have had a myocardial infarction with periodontitis have significantly higher CRP than those with myocardial infarction alone, suggesting that periodontal disease is an independent contributor to systemic inflammation (Kodovazenitis, Pitsavos et al. 2011, Schenkein and Loos 2013).

We have used a rabbit atherosclerosis model because it has the advantage of being a good model for studying therapies to attenuate plaque progression or to reverse atherosclerosis. With modification of the diet, rabbits demonstrate a range of cardiovascular plaque stages that closely resemble those in humans without genetic modification of the animal (Phinikaridou, Hallock et al. 2009, Dornas, Oliveira et al. 2010). The rabbit is also a validated model for experimental periodontitis, which is induced by topical application of a human periodontal pathogen, *Porphyromonas gingivalis* for 6 weeks. (Hasturk, Kantarci et al. 2006, Hasturk, Kantarci et al. 2007). We previously reported simultaneous induction of periodontitis and atherosclerosis in New Zealand White (NZW) rabbits (Jain, Batista et al. 2003). Rabbits with experimentally induced periodontitis exhibited more extensive accumulation of aortic lipids than did periodontitis-free animals (Jain, Batista et al. 2003). In a recently published study (Hasturk, Abdallah et al. 2015), we reported the therapeutic impact of RvE1 on initiation of atherosclerosis in the rabbit model. There were several striking findings that further implicate the role of inflammation in periodontitis and cardiovascular disease, as well as further demonstrating their interaction.

First, it was demonstrated that periodontitis enhanced cardiovascular lesions in the high fat diet model and that oral topical RvE1 prevented both periodontitis, as shown in (Hasturk, Kantarci et al. 2006) and cardiovascular changes (Figure 3). Interestingly, in the absence of periodontitis, oral topical dosing of RvE1 had a significantly greater impact on preventing cardiovascular lesion development. These data show that periodontitis has a measureable negative impact by enhancing systemic inflammation. These findings were confirmed with systemic biomarkers. Serum levels of CRP that were markedly elevated in animals with cardiovascular lesions were markedly reduced in animals protected from developing lesions with RvE1 (Table 2).

These results demonstrate both the potential benefits of lipoxins and resolvins for the prevention and treatment of cardiovascular disease and highlight the importance of inflammation in this process. They also show the significant negative impact of periodontitis as a risk factor that may directly impact the progression of cardiovascular plaque formation.

Conclusions

Data continue to accumulate implicating periodontal disease as an important risk factor in a number of inflammatory systemic diseases. Associations are relatively easy to find in large datasets from national surveys. Proving causal relationships is extremely difficult. After more than 20 years of study, a picture is emerging that suggests that periodontitis has a significant impact on the initiation and outcomes of certain diseases. The weight of the evidence is much greater for some diseases.

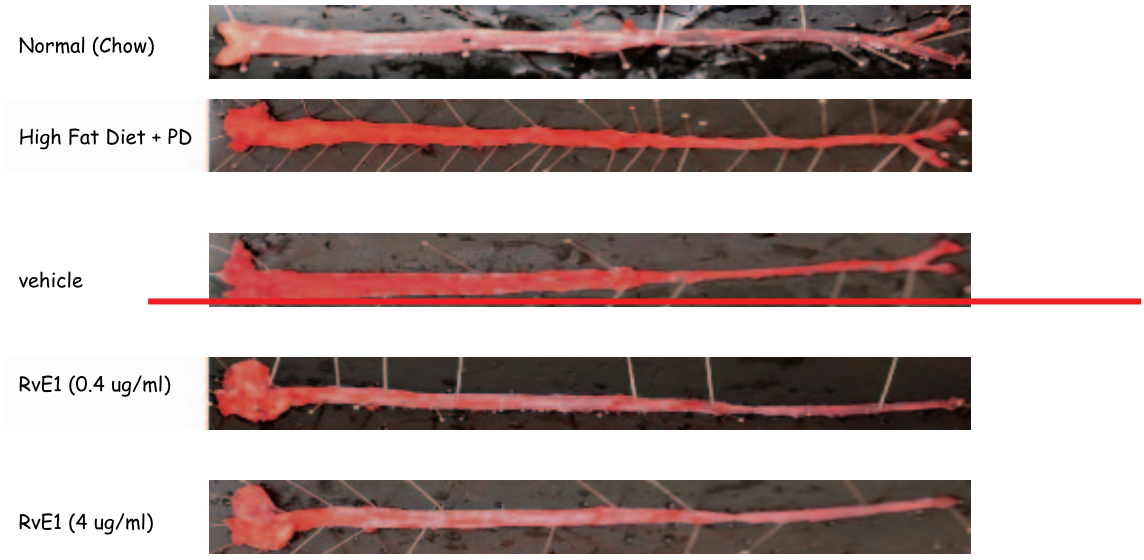

Figure 3. Inhibition of atherogenic changes by RvE1.
Atherogenic changes and periodontitis were induced simultaneously in rabbits with high fat diet and Porphyromonas gingivalis (Hasturk, Abdallah et al. 2015). Aortas were examined en face after staining with Sudan IV. The top panel shows a normal chow aorta compared to a high fat diet fed animal with periodontitis. Note the extensive, red stained lipid deposits. The lower panel shows aortas from animals treated with vehicle and two doses of RvE1, as described in (Hasturk, Abdallah et al. 2015). Note the inhibition of fat deposit at low dose RvE1 that is improved at high dose RvE1.

Table 2. Inflammation induces CRP in serum.

Group	No Treatment	Vehicle	RvE1 (0.4 μg/ml)	RvE1 (4.0 μg/ml)
Normal diet	1.0 ± 0.1			
HFD	7.0 ± 2.1	8.6 ± 0.8	2.2 ± 0.7	1.0 ± 0.5
HFD + PD	14.9 ± 1.8	11.9 ± 0.6	3.1 ± 1.0	1.1 ± 0.7

CRP levels from blood samples collected at 13 weeks are presented. The mean baseline value for all rabbits before the experiment was 1.5 ± 1.1. Rabbit CRP levels were measured using a rabbit-specific ELISA. CRP levels were significantly elevated in all rabbit receiving high fat diet (HFD), but the elevation was significantly higher in animals with periodontal disease (PD). Oral topical treatment with RvE1 (10 μl applied to the gingiva) in animals with PD significantly improved CRP at 0.4 μg/ml and returned to normal levels at 4.0 μg/ml. Interestingly, in the absence of PD, the low dose of RvE1 returned CRP levels to normal.

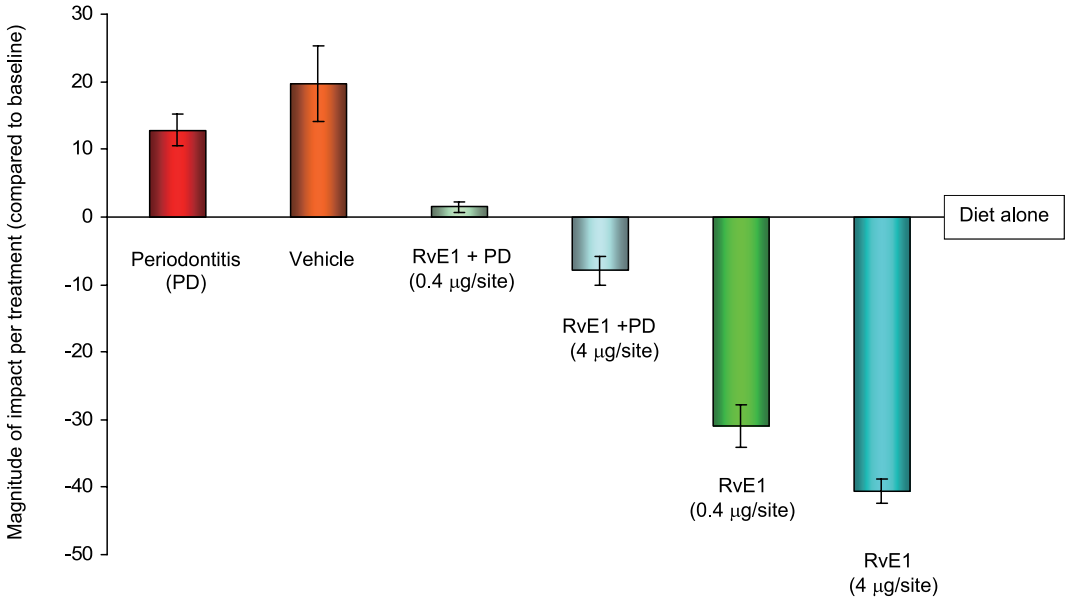

Figure 4. Atherogenic changes compared to high fat diet alone.
Changes in the area of aorta covered by lipid deposits were quantified as percent changes from high fat diet alone (horizontal line). PD increased the area by over 10%, which was greater after vehicle treatment. Low dose treatment of PD with RvE1 brought the area back to diet alone values and high dose RvE1 showed a 20% improvement (PD + 12% to -8%). In the absence of PD, oral topical RvE1 reduced lipid covered area 40-50% suggesting that PD induced inflammation has a direct impact on severity of atherogenic changes in the model.

The mechanism of action for the interaction varies with disease, but in most cases, it is related to inflammation. In some instances (pulmonary disease), the interaction is directly bacterial seeding the lungs with oral bacteria. However, in most diseases, such as diabetes and cardiovascular disease, the role of bacteria is less clear. It is thought that bacteremia plays a role in initiation of systemic inflammation, but it is the inflammation that seems to drive the interaction.

The other aspect of the periodontal disease-systemic disease interaction for which we have little data (with the exception of diabetes) is directionality. Most studies are designed to show, or assume, that periodontitis impacts the systemic disease. Is the converse also true? It is in diabetes (Chapple, Genco et al. 2013), but we know little about increased susceptibility to periodontitis in people with cardiovascular diseases or rheumatoid arthritis.

Clearly, there is much work to be done. However, the promise of new methods to control inflammation to control both periodontitis and inflammatory systemic disease will likely lead to new therapeutic approaches.

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Dental Infection of *Porphyromonas gingivalis* Exacerbates Pathological Progression of Non-Alcoholic Steatohepatitis (NASH)

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Key words: Dental infection, *Porphyromonas gingivalis*, non-alcoholic steatohepatitis, TLR2, inflammasome, Oral Systemic Disease Connection

ABSTRACT

Obesity is becoming a worldwide epidemic. Especially abdominal obesity closely relates to metabolic syndrome including diabetes mellitus, high cholesterol and high blood pressure, which are risk factors for the most dangerous heart attack. It is well accepted that the stress responses caused by "slight chronic inflammation" contribute to the development/progression of metabolic syndrome. Periodontitis is chronic infectious disease. "Slight chronic inflammation" caused by periodontal pathogen like *Porphyromonas gingivalis* (*P.g.*) may deteriorate systemic diseases such as cardiovascular disease, diabetes mellitus and preterm birth. In addition, *P.g.* is detected in atheromatous plaque and placenta with preterm birth, indicating that *P.g.* can enter the circulation and disseminate throughout the body. However there is little study which has reported a relationship between *P.g.* and liver diseases. Fatty liver and non-alcoholic steatohepatitis (NASH) are liver phenotypes of metabolic syndrome. The prevalence is increasing because of the epidemic rise in obesity. Although fatty liver generally has a benign prognosis, it has the potential to progress to NASH, cirrhosis and eventually hepatocellular carcinoma. Therefore, NASH is critical health problem, which is required appropriate prevention and early intervention. Recent our data showed that *P.g.* exacerbated diet-induced NASH via the induction of inflammasome and inflammatory cytokines in the liver. We also demonstrated that detection of *P.g.* in liver from NASH patients were related with advanced fibrosis.

Conclusion : Dental infection of *P.g.* may play an important role in NASH progression. Therefore, preventing and/or eliminating *P.g.* infection by dental therapy may have a beneficial impact on management of NASH.

INTRODUCTION

Obesity is becoming a worldwide epidemic. Today, 2.1 billion people—nearly 30% of the world's population—are either obese or overweight. Especially abdominal obesity closely relates to metabolic syndrome including diabetes, high cholesterol and high blood pressure, which are risk factors for the most dangerous heart attack[1]. Recent studies have focused on "slight chronic inflammation" as common pathological mechanism of the metabolic syndrome. The long-term stress responses caused by "slight chronic inflammation" establish viscous cycle between abnormal metabolism and tissue injury resulting in the development/progression of metabolic syndrome[2].

Periodontitis is chronic infectious disease, which is symptomless in early stage. It is reported that "slight chronic inflammation" caused by periodontal pathogen may deteriorate systemic diseases such as cardiovascular disease, diabetes mellitus, preterm birth and rheumatoid arthritis[3-5]. *Porphyromonas gingivalis* (*P.g.*); one of the most important dental pathogens, is related to both of chronic marginal periodontitis and periapical periodontitis[6,7]. *P.g.* is known to enter the blood circulation and is disseminated throughout the body. *P.g.* DNA is detected in atherosclerotic plaque. However there is little information showing a relationship between *P.g.* and liver diseases[3-5].

Non-alcoholic Steatohepatitis (NASH)

Fatty liver is liver phenotype of metabolic syndrome in adults and children, affecting over 30% of the population in Western countries[8,9]. In Japan, its prevalence is 10%-30% in adults and increasing because of the epidemic rise in obesity and diabetes mellitus[9] It is well known that alcoholic liver injury has pathological progression from fatty liver, steatohepatitis, cirrhosis and hepatocellular carcinoma. In 1980, Ludwig et al[10]. first described non-alcoholic steatohepatitis (NASH) in a series of patients whose liver histology mimicked alcoholic steatohepatitis without history of alcohol abuse. Although fatty liver generally has a benign prognosis, it has the potential to progress to NASH, cirrhosis and eventually hepatocellular carcinoma[8,9,10]. Therefore, NASH is critical health problem, which requires appropriate preven-

tion and early intervention. In 1998, the two-hit hypothesis of NASH pathogenesis was proposed[11]. The first hit involves fat accumulation in the liver as a result of excessive delivery of free fatty acids and imbalance of lipid synthesis and export in hepatocytes. The second hit involves oxidative stress caused by factors that enhance the production of reactive oxygen species[11,12]. Growing evidence indicates that lipopolysaccharides (LPS) originating from the enteric bacteria can act as a second hit[13,14]. *P.g.* is gram negative bacteria and also possess LPS similar to enteric bacteria. However a relationship between *P.g.* and NASH is not well understood. Recently, we demonstrated that *P.g.* exacerbated diet-induced steatohepatitis via the induction of inflammasome and inflammatory cytokines in the liver. Furthermore, the infection of *P.g.* was demonstrated for the first time in the liver[15].

Effects of Dental Infection of *P.g.* on High Fat Diet Induced Sreatohepatitis in Mice

5-week-old male C57BL/6J mice were randomly divided into two groups. One fed a high fat diet (HFD group), the other fed a chow-diet (CD group). After development of fatty liver for 12 weeks of HFD feeding, mice were divided into two subgroups, with and without dental infection of *P.g.*, named HFD-*P.g.* (+) and HFD-*P.g.* (–), respectively. CD-*P.g.* (+) and CD-*P.g.* (–) were also prepared to serve as control. *P.g.* is detected not only in biofilm in periodontal pockets but also as a major bacterium in infected pulp chambers with periapical periodontal diseases[6,7]. Therefore, we applied *P.g.* from pulp chamber where anaerobic conditions suitable for *P.g.* growth were easily established.

In contrast to normal periodontal tissues, all the animals of CD-*P.g.* (+) and HFD-*P.g.* (+) showed total pulp necrosis and periapical granuloma with infiltration of neutrophils and macrophages. *P.g.* was immunodetected in the pulp chamber and in neutrophils and macrophages in the periapical granuloma. Serum LPS was significantly upregulated. These observations indicate that the periapical granuloma is a stable and persistent supply source of the *P.g.* and its products.

Histological examination of the liver showed that no obvious pathological changes were observed in CD-*P.g.* (–) (Fig. 1A) and CD-*P.g.* (+). In HFD-*P.g.* (–), marked steatosis and small foci of Mac2-positive macrophages were observed. In HFD-*P.g.* (+), the amount of fat deposited in hepatocytes was greater than in HFD-*P.g.* (–) (Fig. 1B). Infiltration of macrophages was more prominent. Interestingly, focal fibrosis of the liver was observed only in HFD-*P.g.* (+) (Fig1. C). Spindle cells in the fibrosis area were immunopositive for α-SMA, indicating their myofibroblastic nature (namely hepatic stellate cells, Fig. 1D). Accumulation of collagen around the hepatic stellate cells was revealed by Azan-Mallory staining (Fig. 1E). Analysis of fibrosis showed that most cases in CD-*P.g.* (–) and CD-*P.g.* (+) were in stage 0. In HFD-*P.g.* (–), there were two of stage 2 and four of stage 3. Moreover, HFD-*P.g.* (+) included four of stage 3 and two of stage 4, indicating greater progression of fibrosis in HFD-*P.g.* (+) than in the other experimental group (Table 1). Interestingly, immunohistochemical staining revealed that *P.g.*-positive particles were detected in hepatocytes (Fig. 1F) and Kupffer cells.

Table. Number of animals and stage of fibrosis in liver

	Stage of fibrosis				
	Stage 0	**Stage1**	**Stage2**	**Stage3**	**Stage4**
CD-*P.g.* (–)	6	0	0	0	0
CD-*P.g.* (+)	5	0	1	0	0
HFD-*P.g.* (-)	0	0	2	4	0
HFD-*P.g.* (+)	0	0	0	4	2

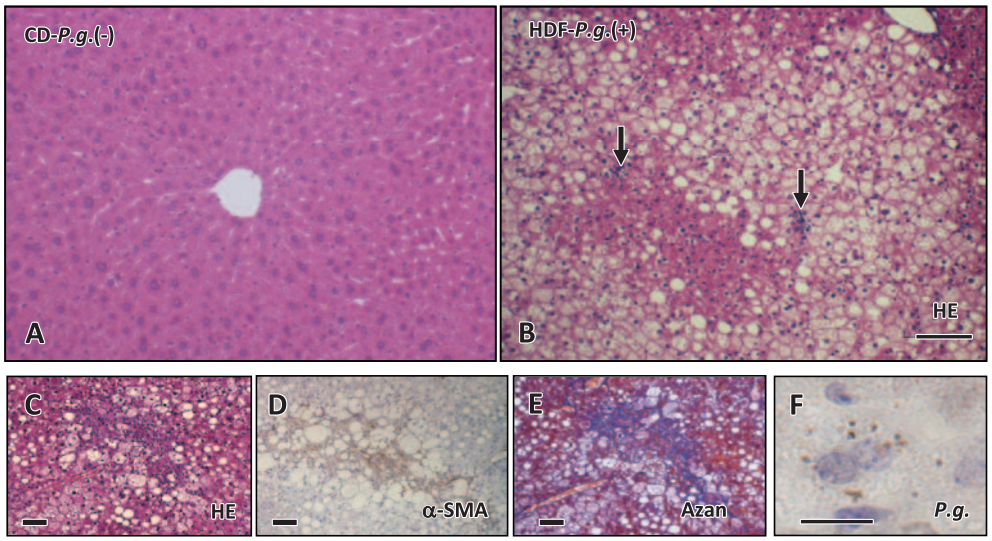

Fig. 1 Dental infection of *P.g.* promotes pathological progression of HFD-induced NASH.
(A) CD-*P.g.* (–) (HE, Scale bar = 10 μm). (B) HFD-*P.g.* (+). Foci of macrophages (arrows) (HE, Scale bars = 100 μm). (C) An area of fibrosis seen in HFD-*P.g.* (+). (D); Immunohistochemistry of α-SMA, (E) Azan Mallory staining, Scale bars = 10 μm) Immunolocalization of *P.g.* in hepatocyte of HFD-*P.g.* (+), Scale bar = 10 μm. *P.g.*; *Porphyromonas gingivalis*, CD; cho diet, HFD; high fat diet. (revised from original article Furusho et al. J Gastroenterol2013: Nov; 48 (11): 1259-70.)

These findings indicate that dentally applied *P.g.* enters the blood circulation, translocates into the liver and accelerates pathological progression of NASH.

Effects of Excessive Fat Accumulation on Inflammasome Activation and Cytokine Production from Hepatocytes

FFAs appear to be the major mediator of excessive fat accumulation in the liver. In patients with NASH, serum FFAs, especially palmitate levels are commonly elevated, which is related to disease severity and considered a potential endogenous danger signal[16]. Therefore we examined molecular mechanisms, in which dental infection of *P.g.* accelerated inflammation and fibrosis in fatty liver using steatotic hepatocytes with palmitate treatment. Palmitate treatment induced fat accumulation (Fig. 2A) and upregulated expression of TLR2 (one of *P.g.*-LPS receptors) (Fig. 2B) in hepatocytes. Steatotic hepatocytes with *P.g.*-LPS promoted expression of proinflammatory cytokines and activated NLRP3-inflammasome (Fig. 2C). Actually TLR2 expression was upregulated in steatotic liver of HFD groups at protein (Fig. 2D) and mRNA levels. Our results lead to two potential mechanisms in which *P.g.*-LPS dramatically upregulated expression of proinflammatory cytokines in steatotic hepatocytes. One is amplification of inflammation through the TLR2-pathway. In steatotic hepatocyte, the sensitivity to *P.g.*-LPS is strengthened by upregulation of TLR2. The second mechanism is activation of the IL-1β pathway through NLRP3 inflammasome. Recently, it has been reported that NLRP3 inflammasome senses obesity-associated danger signals like FFAs and contributes to obesity-induced inflammation and insulin resistance. It leads to Casp-1 cleavage and subsequent activation of IL-1β and IL-18. Csak et al.[17] demonstrated that long term HFD-feeding induced steatohepatitis associated with increased IL-1β production and NLRP3 inflammasome activation.

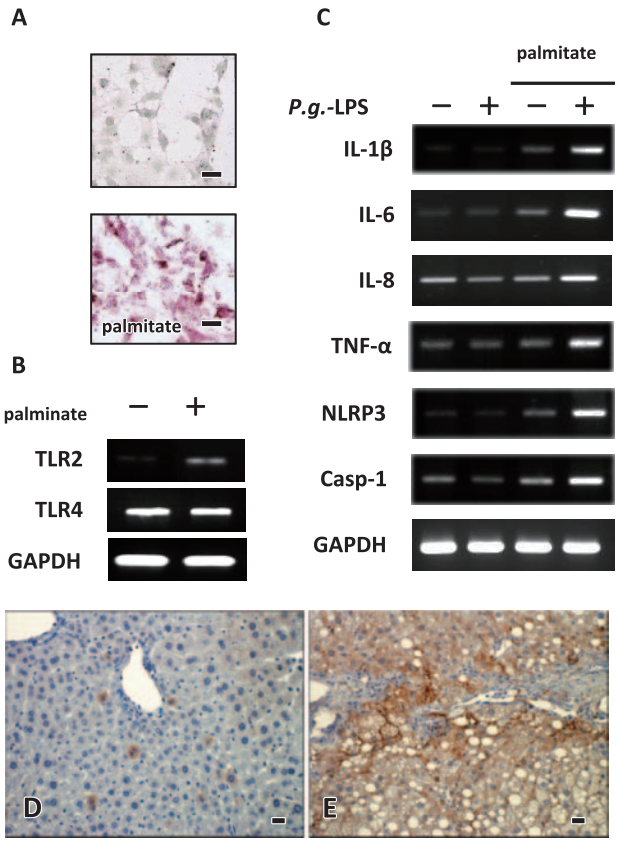

Fig. 2 Free fatty acid promotes inflammasome and LPS-induced proinflammatory cytokine mRNA expression through upregulation of TLR2. Human hepatocyte cell line (Hc3716-hTERT) was treated with 0.4 mM palmitate for 18 hours. (A) Oil Red O staining (Scale bars = 10 μm). (B) mRNA expression of *P.g.*-LPS receptors. (C) mRNA expression of inflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) and inflammasome (NLRP3 and casp-1) in human hepatocytes with and without palmitate treatment at 6 hours after *P.g.*-LPS stimulation. (D) TLR2 expression in CD-P.g. (–). (Scale bars = 10 μm). (E) TLR2 expression in HFD-*P.g.* (+) (Scale bars = 10 μm). (cited from original article Furusho et al. J Gastroenterol 2013: Nov; 48 (11): 1259-70.)

P.g. Infection of NASH Patients and Relation to Clinicopathologic Features.

To immunohistochemically detect *P.g.* in the liver formalin-fixed, paraffin-embedded human liver biopsy samples from patients with NASH were retrieved from the pathological file of Hiroshima University Hospital. *P.g.* was identified as single and aggregated brown particles in hepatocytes (fig. 3). Interestingly, the *P.g.*-positive cases showed significantly higher fibrosis scores than the *P.g.*-negative cases. Now, we are analyzing titer of *P.g.* antibody against type4 FimA fimbriae in serum from NASH patients by ELISA.

Chronic periodontitis, one of dental infection, is among the most prevalent of microbial disease in human and occurs worldwide and the prevalent rate is more than 70%[18]. Recently it is well accepted that control of periodontitis can improve status of systemic diseases including type 2 diabetes and cardiovascular disease[3,4]. There is only one paper indicating that periodontal treatment induces improvement of serum ALT and /or AST[19]. Now we are going to examine periodontal condition of NASH patients in correlation with presence of *P.g.* infection in the liver and to study on the efficiency of dental therapy and/or elimination of liver infection by antibiotics in liver function and condition.

CONCLUSION

In summary, we first demonstrated that the dental infection of *P.g.* exacerbated the pathological progression of NASH from simple steatohepatitis to steatohepatitis with fibrosis through a mechanism that involves synergistic interaction between FFA-induced NLRP3 inflammasome activation and the LPS-TLR pathway. Moreover we confirmed that serum *P.g.* antibody titer and *P.g.* liver infection correlated with NASH patients with advanced fibrosis. Our findings suggest that preventing and/or eliminating *P.g.* infection by dental therapy may have a beneficial impact on NASH.

ACKNOWLEDGEMENTS

The authors express appreciation to Dr. H Hyogo and Dr. K Cyayama in Department of Medicine and Molecular Science and Dr. J Hisatsune and Dr. M Sugai in Department of Bacteriology, Hiroshima University to help and give advices in the present study.

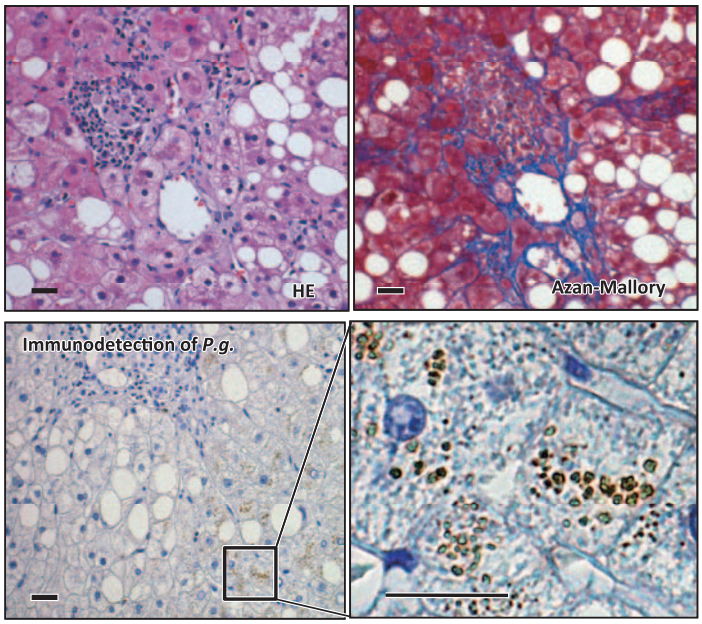

Fig. 3 *P.g.* infection is detected in the liver biopsy specimens from patients with NASH. HE, Azan-Mallory staining, immunohistochemistry for *P.g.*. *P.g.* detected in hepatocytes associated with fibrosis. (Scale bars = 10 μm). (cited from original article Furusho et al. J Gastroenterol 2013: Nov; 48 (11): 1259-70.)

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The Effect of Periodontopathogenic Bacteria, *Porphyromonas gingivalis*, against the Onset of Rheumatoid Arthritis

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ABSTRACT

Periodontitis is the infectious disease in oral cavity caused by the interaction between host and periodontopathogenic bacteria. *Porphyromonas gingivalis* (Pg) is related to periodontitis. As previously reported, Pg also shows the strong correlation with systemic disease as diabetic mellitus (DM), arteriosclerosis, obesity, non-alcoholic steatohepatitis (NASH), cardiovascular disturbance and rheumatoid arthritis (RA). The clarification of Pg effect in the onset of systemic disease is necessary to confirm the etiological findings. RA model mice with Pg infection was established and molecular mechanism involved in the onset of RA by Pg infection was determined.

In order to establish RA mice, SKG mice (CREA Japan) were received i.p. injection of laminarin. Pg was also infected into mice via i.p. injection.

The Pg infected RA mice (Pg-RA mice) showed severe symptoms compared with RA mice without Pg infection in the joint swelling and bone disruption in ankle joint. The infiltration of immune cell and pannus, which was inflammatory dysplasia of RA joint tissue in synovial cell, and a number of osteoclasts were observed. The inflammatory cytokine, anti-cyclic citrullinated peptide antibody in Pg-RA mice were elevated compared with RA mice. Furthermore, the mRNA expression of osteoclastogenesis related gene was induced in the bone marrow cell from Pg-RA mice.

These findings show that Pg infection has potential for RA exacerbation.

INTRODUCTION

Periodontitis is caused by the interaction between periodontopathogenic bacteria, such as Pg, *Tarnnarella forthsia* and *Treponema denticola*, and host immune response. The excessive interaction may cause the destruction of supportive periodontal tissue and loss of teeth[1]. Pg is the gram negative and obligately anaerobe classified into the red complex group by Socransky[2]. Pg possesses some kinds of virulence factors to induce inflammation in periodontal tissue (Fig. 1). Outer membrane proteins (OMPs), fimbriae, and gingipain (KGP, RGP) to induce inflammation in gingival tissue for progression of periodontitis[3-5]. Among various pathogenic factors, lipopolysaccharide (LPS) in the outer membrane of Pg is also a strong modulator of inflammation[6]. Recently, the association periodontitis and systemic dis-

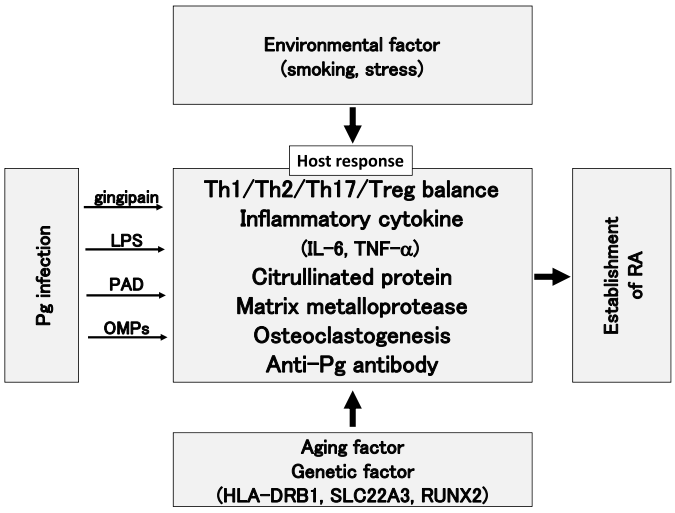

Fig. 1. Pg infection on RA establishment
RA is an autoimmune disease caused by some aging, genetic, and environmental factors. In addition to these factors, Pg infection is strongly related with RA. Pg possesses diverse pathogenic factors to activate local and systemic immune response. As the result of excess immune response, some characterized clinical symptoms of RA appears in local tissue.

eases such as diabetes mellitus (DM), arterial sclerosis, nonalcoholic steatohepatitis (NASH), low weight birth and rheumatoid arthritis (RA) were reported[7-11]. The locally induced inflammatory cytokine caused by Pg direct challenge or Pg infection into blood stream, bacteremia, may exacerbate the onset of systemic disease[12]. RA is a systemic autoimmune disease characterized by chronic inflammation of the diarthrodial joints leading to bone erosions and progressive disability[13]. There is a general agreement of two hit theory that RA develops as a result of interaction between susceptibility genes, hormonal and environmental factors, such as smoking, silica, female hormone, and periodontitis[14]. Most of the genetic studies on RA have been focused on the Human Leucocyte Antigen (HLA) region, and PADI4 gene that codifies for the peptidyl arginine deiminase type IV enzyme[15]. As the result of the mutation of HLA and PADI4, the elevation of anti-citrullinated protein antibody (ACPA) occurs following the increase of citrullinated protein[16]. The production of ACPA in serum can be the important diagnostic marker of RA in common with rheumatoid factor which is the autoantibody of IgM against Fc portion of IgG. There is the report about the correlation RA and periodontitis. Especially, production of citrullinated protein by peptidylarginine deiminases (PAD) derived from Pg (PgPAD) shows the strong involvement in the onset of RA[17]. The citrullination is a post-translational modification of arginine residues, mediated by the family of PAD. The mechanism of generation of CP by PgPAD is different from endogenously produced CP. The fragmented target protein, such as fibrinogen and alpha-enolase, after digestion by Pg produced arginine specific enzyme, gingipain (RGP) is targeted the citrullination of c-terminal arginine[18]. The increase of CP and ACPA by PgPAD also can be the cause of RA. Therefore, there is a common issue to cooperate and overcome for the treatment of RA and periodontitis. As for the correlation between periodontitis and RA in clinical study, the non-surgical treatment of periodontitis reduces the severity of RA in patients treated with or without tumor necrosis factor inhibitors[14]. The elevation of inflammatory cytokine, such as IL-6 and TNF-α, and matrix metalloprotease (MMP) 3 are the major clinical parameter of RA. These factors may communicate the progression of periodontitis via blood stream. Therefore, the use of cytokine inhibitor, Infliximab (anti-TNF-α neutralization antibody), Etanercept, (anti-TNF-α receptor antibody), Adalimumab (anti-TNF-α neutralization antibody), Tocilizumab, (anti-IL-6 receptor antibody), IL-6 receptor, results in the improvement of periodontitis condition by suppressing the inflammation in periodontal tissue[19]. Previously, to examine whether induction of periodontitis affects the progression of experimental arthritis in vivo, mice immunized with CII received orally inoculations of Pg every other day. Interestingly, periodontitis induced by Pg accelerated the onset of arthritis in addition to periodontal bone loss in mice. The analysis of T cell population in joint-draining lymph node with Pg infection showed the activation of Th17. Furthermore, the Pg stimulation activated the bone marrow derived dendritic cells (BMDCs) to produce cytokine affecting T cell differentiation, such as IL-1β, IL-6, TNF-α via toll like receptor 2 signaling[20]. Although there are some reports focused on the immune response against periodontopathogenic bacteria in RA model mice, the effect of Pg infection in the bone resorption as one of the progressed RA symptom. However, it is important to clarify the involvement of Pg infection in the osteoclastogenesis in advanced RA for treatment of systemic disease. Here, we aimed to elucidate the local and systemic immune response in the progression of RA by establishing Pg infected SKG mice as RA model.

RESULT

Firstly, RA model mice was established by injecting laminarin, which was the group of β-glucan from *Laminaria digitata*. The joint swelling was monitored established by Sakaguchi's arthritis score[21]. As shown in Fig. 2, the single injection of laminarin induced RA symptom in 20 weeks. The Pg infection induced the joint swelling in laminarin injected RA mice. The sever tissue destruction and forming of pannus were observed by microCT and immunohistochemical staining in Pg injected RA mice. The production of MMP- 3 and anti-CCP IgG in serum were statistically increased in the Pg injected RA mice. The increase of CP and Pg derived protein were detected in ankle joint tissue of Pg injected RA mice. The production of IL-1β, IL-2, IL-6, and CXCL1 in serum of Pg injected RA mice was increased compared with RA induced mice. The osteoclast differentiation of bone marrow derived mononuclear cell was strongly induced in Pg injected RA mice. Furthermore, the induction of osteoclastogenesis was supported by the induction of mRNA expression of RANK, OSCAR, TRAF6, c-Fos, NFATc1, CatK, MMP-9, and DC-STAMP.

CONCLUSION

These findings might the involvement of Pg in the exacerbation of RA. Further studies will be needed in order to clarify the detail of the correlation between periodontitis and RA.

ACKNOWLEDGEMENT

This research was supported by a Grant-in-Aid for the Encouragement of Young Scientists (B) (22792085) from the Japan Society for the Promotion of Science.

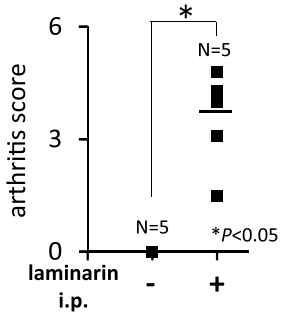

Fig. 2. The assessment of joint swelling after 20 weeks.
The SKG mice were received i.p. injection of laminarin (Sigma, 10 μg/mouse) at day 0. Mice were divided into 2 groups (no injection, laminrin single injection). The joint swelling was assessed by Sakaguchi's arthritis score after 20 weeks of LA injection. Three independent experiments of all groups were analyzed by Mann-Whitney U test.

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Periodontal Disease as a Possible Risk Factor for Alzheimer's Disease

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ABSTRACT

Periodontal disease is known to cause systemic spread of chronic inflammation and exacerbate lifestyle-related diseases such as ischemic heart disease, diabetes mellitus, and obesity, while the inflammatory response plays a major role in the development of neurodegenerative conditions such as Alzheimer's disease (AD). Mild systemic inflammation has been reported to increase the risk of AD. Chronic inflammation in periodontal disease and periodontal disease-related bacteria are transmitted to the brain via an unknown mechanism, possibly exacerbating AD. In this super-aging society, measures for prevention of periodontal disease will become increasingly important.

INTRODUCTION

Rather than systemic diseases being risk factors for periodontal disease, periodontal disease has been shown to cause systemic diseases, including lifestyle-related diseases. To date, periodontal disease has been reported to be a risk factor for diabetes mellitus (DM), cardio- and cerebrovascular diseases, aspiration pneumonia, premature and low birth weight infants, bacterial endocarditis, glomerulonephritis, arthritis, and palmoplantar pustulosis[1-5]. The following three pathways are assumed to be the mechanisms of the systemic spread of periodontal disease: direct action of bacterial bodies and toxins of periodontal disease-related bacteria at a local periodontal site that spread to target organs through a hematogenous route or the respiratory tract[4]; actions of inflammation-inducing substances such as cytokines, produced by the inflammatory response within periodontal tissue or by the immune response, that spread hematogenously to the target organs[5]; and a pathway that results in intracerebral spread through the nervous system[6,7]. There are various data on intravascular infiltration of periodontal disease-related bacteria and their spread to target organs, but the mechanism by which they affect diseases is not fully understood. On the other hand, the inflammatory response is known to play a major role in the progression of cerebrovascular disorders and dementia, conditions that often occur in the elderly[8-10]; however, the effect of periodontal disease is not fully understood.

Taking results of other studies into consideration, we discuss here our most recent results of analysis of the correlation between periodontal disease and Alzheimer's disease (AD) in a mouse model in this study.

Current status of AD in Japan

More than 30 million people in Japan are over 65 years of age. A study group of the Ministry of Health, Labour and Welfare (2013) showed that the number of people with dementia was 4,620,000, with another 4 million people in the general population estimated to have dementia. This number of people with dementia is expected to increase in the future. Sixty to seventy percent of people with dementia have AD, which is a serious problem in an advancing aging society such as that in Japan. There is still no effective method for prevention of AD or a fundamental method for treatment[11].

Inflammation and AD

In addition to aging and genetic mutation, AD is caused by accumulation of amyloid β protein (Aβ) due to intracerebral inflammation[12]. Aβ deposits also cause inflammation, which results in the progression of synapse disorders and neuronopathy. In recent years, mutations of the *TREM2* gene, which controls the inflammatory response, have been found in patients with AD, renewing the importance of the inflammatory response in the development of AD[13]. Chronic inflammation is also thought to play an important role in the development of central nervous system (CNS) diseases. The long-term use of non-steroidal anti-inflammatory drugs is known to prevent the occurrence of neurodegenerative disease. The effectiveness of these drugs for delaying the progression of neurodegenerative disease has been shown by an epidemiologic study and an animal experiment[14]. The immune system of the CNS is extremely simple and is not acquired. Accordingly, the immune response is served by the innate immune system.

Microglia are cells of the macrophage system that have a phagocytic capacity that plays a central role in the intracerebral innate immune response. Microglia digest Aβ that has accumulated in the brain and remove it from the brain. These cells produce cytokines such as active oxygen, interleukin (IL)-1, IL-6, tumor necrosis factor-α (TNF-α), i.e., an inflammatory response producing molecules that are known to promote neurodegeneration in AD[15,16]. On the other hand, they also produce anti-inflammatory molecules such as IL-4 and IL-10, which are thought to have a neuroprotective role in addition to controlling the inflammatory response[17]. Therefore, microglia are important cells in the control of AD status.

Apart from the exacerbation of AD status due to intracerebral inflammation, mild systemic inflammation has been reported to reduce cognitive function and hippocampal capacity and increase the risk of AD[18,19,20]. Inflammation has been thought to spread through the circulatory system and CNS. Increased amounts of inflammatory mediators in the blood are transmitted to the

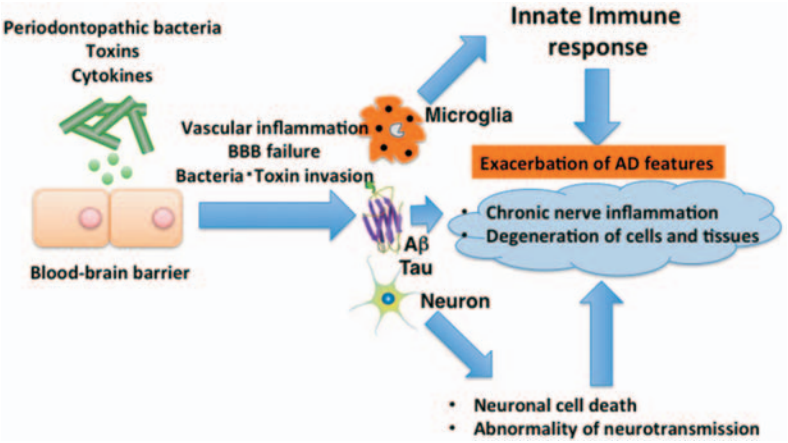

Fig. 1 Possible mechanisms by which periodontitis induced by bacterial infection exacerbate features of Alzheimer's disease.

brain and may activate microglia in the brain. TNF-α level is increased in the blood of patients with AD and reportedly correlates with reduced cognitive function[21,22].

Periodontal disease and AD

As mentioned earlier, chronic inflammation within peripheral organs might play a role in exacerbation of the molecular pathogenesis of AD. One such inflammatory condition is periodontal disease. The inflammatory response that occurs in periodontal disease is known to be involve in the development of various diseases, such as arteriosclerotic disease, DM, and obesity, and the incidence of premature and low birth weight infants[1,2,23,3,24,4,5]. Periodontal disease has also been reported to be involved in cerebral abscess formation[25]. Periodontal disease-related bacteria spread systemically through the blood vessels and respiratory tract, suggesting their possible direct effects on target organs. In addition, inflammatory mediators such as cytokines, which are produced in local periodontal tissue, are carried hematogenously to target organ and are thought to worsen the inflammatory response.

There have been interesting reports on the correlation between AD and periodontal disease. *Porphyromonas gingivalis*, a periodontal disease-related bacterium, was found at high frequency in autopsied brain tissues of patients who died of AD; however, it was not found in normal human brain tissues[26]. These finding suggest that said the bacteria spread hematogenously into the brain. *P. gingivalis* is a gram-negative anaerobic bacillus that possesses various toxins including lipopolysaccharide. Accordingly, it is known to cause a strong inflammatory response. In addition, an interesting finding is that periodontal disease-related bacteria of the *Treponema* genus were found in the trigeminal ganglion, brainstem, and cerebral cortex: with a particularly high frequency in patients with AD[6]. These findings suggest that periodontal disease-related bacteria can be directly transmitted to the brain and cause inflammation. The mechanism (hypothesis) of AD exacerbation due to periodontal disease is shown in Figure 1. The mechanism should be analyzed in detail in the future. However, it is unlikely that AD is induced only by an inflammatory response due to periodontal disease and periodontal disease-related bacteria. Inflammatory responses are thought to aggravate the molecular level of AD, cause an earlier onset, worsen the degree of cognitive disorders, and cause more rapid progression, suggesting its action in modifying the disease status. The long-term use of anti-inflammatory drugs has been suggested to reduce the risk of AD onset[27].

Periodontal disease is the main cause of tooth loss; however, some reports have shown a correlation between tooth loss and AD. Tooth loss may be a risk factor for AD[28,29]. Tooth loss reduces chewing function, resulting in reduced cerebral blood flow and possibly leading to reduced cognitive function. However, tooth loss itself is often not accompanied by a chronic inflammatory response, suggesting that the effects of tooth loss are not necessarily the same as those of periodontal disease. Oue et al. found that cognitive function was reduced by tooth removal in AAP transgenic mice, but that there was no effect of tooth removal on the molecular pathology of AD[30]. On the other hand, when we induced periodontal disease in the same mice, we found that intracerebral Aβ deposits increased and that the intracerebral inflammatory response was enhanced in addition to reduced cognitive function. Both periodontal disease and tooth loss reduce cognitive function, but their molecular mechanisms are thought to differ.

CONCLUSION

All organisms survive by consuming food; chewing function is therefore very important. This function not only supports life but also might be important for maintenance of cognitive function. In this modern aging society, prevention of periodontal disease and maintenance of oral cavity function will become increasingly important.

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New Paradigm for the Link between Periodontitis and Systemic Diseases

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Key words: Periodontal medicine, *Porphyromonas gingivalis*, Gut microbiota, Dysbiosis, Endotoxemia, Systemic inflammation

ABSTRACT

Periodontitis has been implicated as a risk factor for metabolic disorders such as type 2 diabetes and atherosclerosis. Hypothesized underlying mechanisms by which periodontitis affect these diseases include a disseminated bacteremia from the periodontal plaque bacteria and/or elevated circulating inflammatory cytokines that are produced locally in the inflamed gingival tissues. However, there is no direct evidence that either a periodontal bacteria-associated bacteremia and/or periodontitis tissue-derived inflammatory cytokines are involved in the course of these systemic diseases. Recent evidence suggests that the gut microbiome plays an important role not only in metabolic homeostasis but also in immune and inflammatory responses. We hypothesize that swallowed oral bacteria disrupt gut microbiota leading to a metabolic endotoxemia seen in obesity and an increase in the incidence and progression of metabolic disorders. To test this hypothesis, C57BL/6 mice were orally administered *Porphyromonas gingivalis*, a representative periodontopathic bacteria. We demonstrate that *P. gingivalis* oral administration induces change of gut microbiota, reduced gene expression of tight junction protein in the ileum, and endotoxemia. The endotoxemia is considered to be induced by the changes of gut microbiota rather than periodontal inflamed surface area. These results provide a new paradigm for the interrelationship between periodontal diseases and systemic diseases.

INTRODUCTION

A large variety of microbes colonize our body surfaces, and our oral cavity and gut lumen are no exceptions (Structure, function and diversity of the healthy human microbiome 2012). These microbes are designated as commensals since the interactions between bacteria and epithelial surfaces are important for homeostasis. Accordingly, disruption in the balance of commensal microbes (dysbiosis) can result in a variety of metabolic and autoimmune diseases (Cho and Blaser 2012).

Periodontal disease is a chronic inflammatory disease resulting from dysbiosis of oral microbiota. Epidemiological studies indicate its association with increased risk of various diseases such as diabetes, atherosclerotic vascular diseases and rheumatoid arthritis. It is possible that common disease susceptibilities and risk factors could explain the association between these diseases. Although direct evidence is still lacking, causal mechanisms proposed include endotoxemia, proinflammatory cytokines and molecular mimicry (Cullinan and Seymour 2013).

Interestingly, the diseases reported as affected by periodontal disease are often described in association with dysbiosis of the gut microbiota. Under physiological conditions, bacteria in the intestine are commensal and mediate food digestion, strengthen the immune system, and prevent pathogens from invading tissues and organs. However, once the balance of commensals is disrupted and detrimental bacteria become predominant, noxious agents such as bacterial toxins and metabolites damage the gut epithelial wall. These are then absorbed into systemic circulation through the disrupted epithelium, resulting in impairment of various tissues and organs such as the liver, heart, kidney, pancreas and blood vessels (Clemente et al. 2012).

Orally administered live bacteria have been shown to affect the composition of the gut microbiota, as evidenced by oral probiotics (Delzenne et al. 2011). The compositions of the oral microbiota and gut microbiota are taxonomically distinct (Koren et al. 2011). This suggests that if sufficient numbers of oral bacteria reach the intestine, these bacteria could affect the composition of gut microbiota. The alteration of the gut microbiota by oral bacteria is an interesting supposition that could explain causal mechanisms of systemic diseases resulting from periodontal diseases. Therefore, we will consider our recent observations and issues of traditional mechanistic hypotheses to inform a discussion of systemic diseases potentially associated with both periodontal disease and gut microbiota dysbiosis.

New insights into the mechanisms linking periodontal disease and systemic disease

Most diseases linking periodontal disease also seem to be affected by dysbiosis of the gut microbiota (Fig. 1) (Belkaid and Hand 2014; Cho and Blaser 2012; Clemente et al. 2012; Fukuda and Ohno 2014). Decreased gut barrier function and subsequent endotoxemia, imbalance of gut immune function, and adverse effects of bacterial metabolites are considered underlying mechanisms,

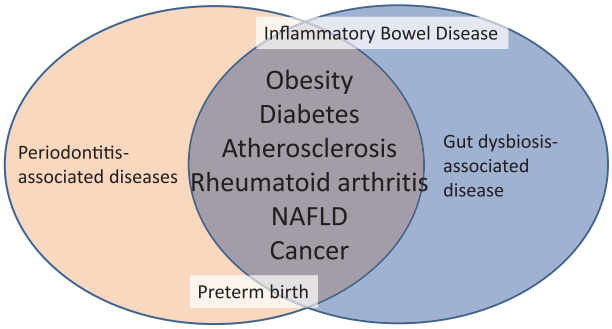


Figure 1. Association between various diseases and periodontitis or dysbiosis of gut microbiota
A number of diseases are reported to be associated with both periodontitis and dysbiosis of gut microbiota.

although details of these remain to be elucidated.

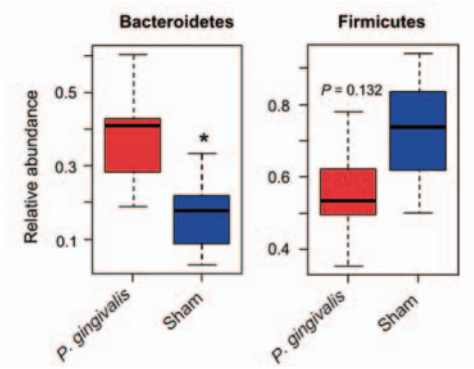
It is well known that large amounts of bacteria live in the saliva of healthy individuals. In patients with severe periodontitis, the concentration of *P. gingivalis* can reach 10^6/ml (Boutaga et al. 2007; Saygun et al. 2011; von Troil-Linden et al. 1995). Since the proportion of *P. gingivalis* in the oral flora is estimated to be 0.8% (Kumar et al. 2006) and humans produce 1-1.5 L of saliva a day, patients with severe periodontitis could be swallowing 10^{12} to 10^{13} (10^9 to 10^{10} as *P. gingivalis*) bacteria per day. Given that the bacterial flora of the oral cavity is quite different from that of the gut, it is possible that a large amount of swallowed oral bacteria could alter the gut flora.

In support of this idea, the effect of oral bacteria on the gut microbiota has recently been shown in patients with liver cirrhosis (Qin et al. 2014). The study revealed a major change in the gut microbiota in patients with liver cirrhosis occurring because of a massive invasion of the gut by oral bacterial species. The correlation in disease severity with the abundance of the invading species suggests that they may play an active role in pathology.

Effect of oral administration of *P. gingivalis* on metabolic change and gut microbiota

To investigate this hypothesis, changes in the gut microbiota, insulin and glucose intolerance, and levels of tissue inflammation were analysed in mice after oral administration of *P. gingivalis* strain W83 twice a week for five weeks (Arimatsu et al. 2014). Pyrosequencing of ileum contents revealed that populations of Bacteroidales were significantly elevated in *P. gingivalis*-administered mice, which coincided with increases in insulin resistance and systemic inflammation (Fig. 2). In *P. gingivalis*-administered mice, blood endotoxin levels tended to be higher, whereas gene expression of tight junction proteins in the ileum decreased significantly. Interestingly, the expression of genes coding for intestinal alkaline phosphatase (Akp3) in the small intestine were both downregulated in *P. gingivalis*-administered mice compared with sham-administered mice. It was demonstrated that a defect in intestinal alkaline phosphatase was associated with high-fat diet-induced metabolic syndrome, and endogenous and orally supplemented IAP inhibited endotoxin absorption as well as reversed metabolic syndrome in mice (Kaliannan et al. 2013).

Analysis of gut microbiota by 16S rRNA sequencing



*p < 0.05, Mann-Whitney U-test

Figure 2. Comparison of the gut microbiota between *P. gingivalis*-administered and sham-administered mice by 16S rRNA sequencing analysis
Relative abundances of each bacterial group in Phylum level are indicated by boxplot.

Gene expression profiles revealed that expression of proinflammatory genes were upregulated, whereas the genes that improve insulin sensitivity were downregulated in the adipose tissue of *P. gingivalis*-administered mice compared with sham-administered mice.

In the liver, oral administration of *P. gingivalis* also led to increased mRNA expression of proinflammatory cytokines and decreased mRNA expression of molecules having potentially anti-inflammatory properties.

In the other direction, the possibility that dysbiosis of gut microbiota affects periodontal tissue destruction has also been investigated. Dextran sodium sulphate (DSS)-colitis is a well-known mouse model of inflammatory bowel disease with changes in gut microbiota (Kim and Berstad 1992; Ohkawara et al. 2002). Inflammatory bowel disease is considered to be a result of an imbalance of the gut microbiota, epithelium, and immune system (Braun and Wei 2007). Although DSS-colitis may not have a direct relationship with periodontal tissue homeostasis, enhanced alveolar bone resorption in mice with DSS-colitis was shown irrespective of oral bacteria manipulation (Oz and Ebersole 2010). These results imply a change in the gut microbiota or its effects on inflammation induced bone resorption. Feeding mice with a diet containing n-3 polyunsaturated fatty acid (n-3 PUFA)-rich fish oil prevented *P. gingivalis*-induced alveolar bone resorption compared with feeding a diet containing saturated fatty acid-rich corn oil. Furthermore, n-3 PUFA-containing fish oil prevented *P. gingivalis*-induced alveolar bone resorption compared with corn oil in mice (Kesavalu et al. 2007). Since n-3 PUFA is shown to have a favourable effect on gut microbiota (Yu et al. 2014), it is suggested that the suppressive effect of n-3 PUFA on alveolar bone resorption could be attributed to the suppression of systemic inflammation through gut microbiota modulation.

These results provide a new paradigm on the interrelationship between periodontitis and systemic diseases.

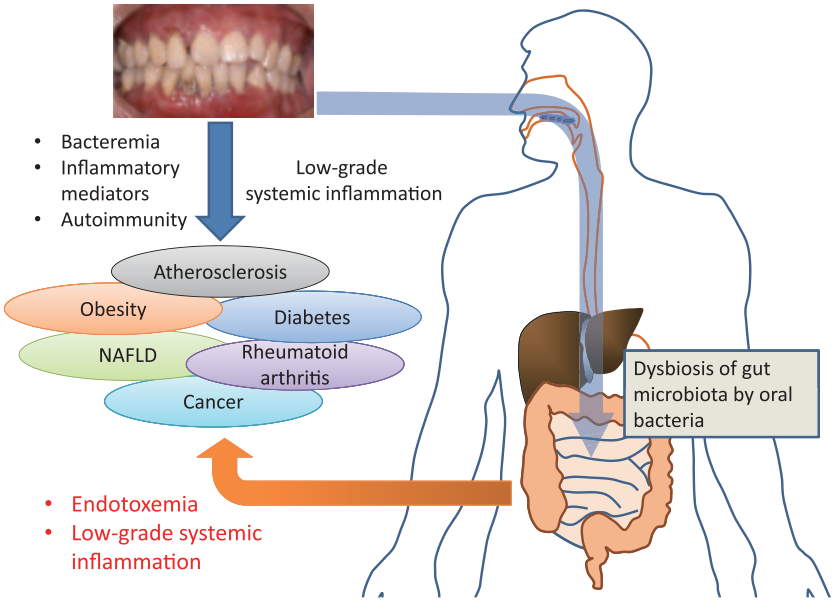


Figure 3. New insights into the mechanisms linking periodontal disease and systemic disease
In the existing hypotheses, bacteria and/or bacterial products from dental plaque invade gingival tissue through ulcerated sulcular epithelial linings of periodontal pockets and then disseminate into systemic circulation.
In the novel hypothesis, endotoxemia is induced by dysbiosis of gut microbiota and concomitant impairment of gut barrier function that is caused by swallowed periodontopathic bacteria.

CONCLUSIONS

It has become evident that oral administration of *P. gingivalis*, a periodontopathic bacterium, induces an alteration of the gut microbiota and an elevation of blood endotoxin levels (Fig. 3). These results are similar to findings observed in either diet-induced obese mice or genetically modified obese mice and diabetic model mice. Accumulating evidence suggests that dysbiosis of the gut microbiota is associated with an increased risk of diabetes, atherosclerosis, NAFLD, obesity, and rheumatoid arthritis. Our study showing that swallowing large amounts of oral bacteria alters the gut microbiota provides a rationale for the biological basis of a causal association between periodontal disease and systemic diseases that cannot be explained by existing hypotheses.

Further studies are needed to determine whether oral bacteria other than *P. gingivalis* can induce similar changes in gut microbiota and metabolism to identify bacterial components responsible for gut microbiota changes and to determine gut bacteria that become pathogenic due to the influx of oral bacteria. Most importantly of all, research is needed to clarify whether the gut microbiota of patients with periodontal disease is different from those of periodontally healthy subjects. Exploration of the gut microbiota of these patients could elucidate a potential association with those of patients with systemic diseases.

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Session III

Host-Microbe Interaction on Health and Diseases: —Frontiers in Basic Clinical Research—

Molecular Basis Involved in Autophagy-Mediated Clearance of Intracellular Pathogens

Hiroshima University

K. Harada-Hada and T. Kanematsu

Exploring the Host Mechanisms that Impact RNA Virus Replication in the Cell Nucleus

Kyoto University

K. Tomonaga

Bacteriotherapy for Inflammatory Bowel Diseases

Keio University School of Medicine

T. Kanai

Role of Dental Science for the Development of Mucosal Immunology and Vaccine

The University of Tokyo

H. Kiyono

Molecular Basis Involved in Autophagy-Mediated Clearance of Intracellular Pathogens

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Key words: autophagy, bacteria, innate immunity, LC3, GABARAP, PRIP

ABSTRACT

Autophagy can be induced by several stressors including nutrient deprivation to immune signaling, resulting in the degradation of cytoplasmic materials. Autophagy is also an intrinsic host defense system that recognizes and eliminates invading bacterial pathogens. A research area on autophagy has begun to focus on its role in inflammation and the immune response. Microtubule-associated protein 1 light chain 3 (LC3), used as a marker for autophagy, is tightly associated with the autophagosomes, and γ-aminobutyric acid receptor-associated protein (GABARAP) reported to be implicated in autophagosome maturation. We have identified a LC3- and GABARAP-binding molecule, phospholipase C-related catalytically inactive protein (PRIP) that was originally identified as an inositol trisphosphate-binding protein. Here, we investigated the involvement of PRIP in the autophagic elimination of pathogenic microorganism in infecting mouse embryonic fibroblasts (MEFs). We infected *Salmonella typhimurium* or *Staphylococcus aureus* into *Prip*-knockout (KO) and wild-type MEFs and observed the process of the autophagic elimination of the bacteria. We observed that the proliferation of bacteria was significantly increased in the *Prip*-KO MEFs compared with the wild-type MEFs. Furthermore, autophagic flux and acidification of autophagosomes were significantly inhibited in *Prip*-KO MEFs, as determined by time-lapse image analysis performed using mRFP-GFP-tagged LC3. These data suggested that PRIP is required for the fusion between bacteria-containing autophagosome-like vacuoles and lysosomes. PRIP is a novel modulator in the regulation of the innate immune system in non-professional phagocytic host cells.

INTRODUCTION

Autophagy, an evolutionarily conserved intracellular catabolic pathway in eukaryotic cells, delivers intracellular materials, such as damaged cytosolic components, into the lysosomes for degradation. Autophagy has also been shown to function as a mechanism of intracellular pathogen sensing, resulting in the elimination of invading pathogens by targeting them to the lysosome. Therefore, defects in autophagy can lead to increased susceptibility to infection (Deretic et al., 2013). In the autophagy pathway, a part of the cytoplasm is sequestered by autophagosomes, which in mammals are double-membrane vacuoles characterized by the presence of specific structures containing microtubule-associated protein 1 light chain 3 (LC3), a homologue of yeast autophagy-related protein 8 (Atg8) (Kabeya et al., 2000). Mammals have at least six Atg8 orthologs that can be broadly classified into two large subfamilies: LC3s and γ-aminobutyric acid (GABA) receptor-associated proteins (GABARAPs). GABARAPs were initially identified as trafficking modulators for transmembrane receptors, such as the $GABA_A$, κ-opioid and transferrin receptors, from the Golgi to the plasma membrane (Leil et al., 2004), and subsequently as autophagy modulators (Shibutani & Yoshimori, 2014). Functional studies in mammalian cells have placed GABARAPs downstream of LC3 during autophagy (Wang et al., 2015; Weidberg et al., 2010). The multiple steps of autophagy generally consist of the formation of a phagophore, which is the membrane precursor of the autophagosome; the elongation and closure of the membrane; and the maturation of autophagosomes by fusion with lysosomes, resulting in the formation of autolysosomes, thus acquiring an acidic compartment for degradation (Deretic & Levine, 2009).

We have recently reported that phospholipase C (PLC)-related catalytically inactive protein (PRIP) is a modulator for canonical autophagy (Umebayashi et al., 2013) and is involved in the elimination of *Staphylococcus aureus* by autophagy (Harada-Hada et al., 2014). PRIP was originally identified as a D-myo-inositol 1,4,5-trisphosphate-binding protein in rat brain (Kanematsu et al., 1992), and has a domain organization similar to that of phospholipase-C (PLC) δ1, but lacks enzyme activity of PLC (Kanematsu et al., 1996, 2000). PRIP has several binding partners: inositol 1,4,5-trisphosphate (Harada et al., 2005; Kanematsu et al., 1992, 1996, 2000; Takeuchi et al., 2000), GABARAP (Kanematsu et al., 2002; Mizokami et al., 2007), $GABA_A$ receptor β subunit (Kanematsu et al., 2006, 2007), the catalytic subunit of protein phosphatase 1 and protein phosphatase 2A (Kanematsu et al., 2006, 2007; Yoshimura et al., 2001; Terunuma et al., 2004), phospho-Akt/protein kinase B (Fujii et al., 2010), and LC3 (Harada-Hada et al., 2014; Umebayashi et al., 2013). The functional aspects of PRIP have been characterized using PRIP knockout (*Prip*-KO) mice.

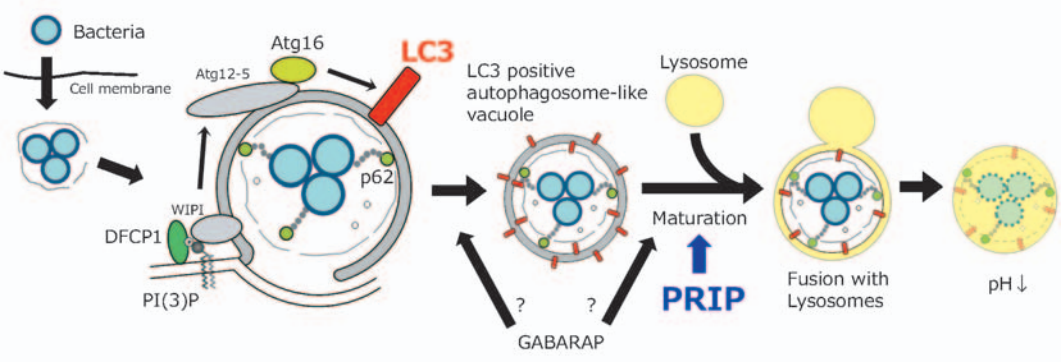

Figure 1. A possible mechanism in PRIP-mediated autophagic elimination of bacteria
Invading bacterial pathogens are sequestered by autophagosome-like vacuoles decorated with phosphatidylinositol 3-phosphate [PI(3)P], double FYVE domain-containing protein 1 (DFCP1), and WD repeat domain phosphoinositide-interacting proteins (WIPI). The LC3-positive autophagosome-like vacuoles are fused with lysosome, and autolysosomes are formed, resulting in elimination of bacteria. PRIP may regulate this maturation process of autophagy, and GABARAP may be involved in the enclosing and/or maturation process together with PRIP, a binding partner of GABARAP.

Accumulation of *S. typhimurium* and *S. aureus* in LC3-positive autophagosome-like vacuoles are observed in *Prip*-KO MEFs compared with wild-type MEFs

To investigate whether PRIP affects proliferation of bacteria in infecting host cells, we used MEFs prepared from *Prip*-KO and wild-type mice to examine mechanisms of PRIP-mediated elimination of *Salmonella typhimurium*, a gram-negative bacterial pathogen that causes human foodborne illness worldwide by infecting both mammalian intestinal epithelial cells and macrophages, and *S. aureus*, a gram-positive bacterial pathogen that causes serious diseases including pneumonia, endocarditis, and osteomyelitis, in addition to wound infection, by autophagic pathway. Either *S. typhimurium* or *S. aureus* were infected into MEFs that were transfected with mRFP-LC3. The number of *S. typhimurium* or *S. aureus* was increased in the LC3-positive autophagosome-like vacuoles in *Prip*-KO MEFs compared with wild-type MEFs.

Inhibition of autophagic flux and acidification in *Prip*-KO MEFs

We next monitored the autophagy maturation using a tandem fluorescent-tagged LC3 (RFP-GFP-LC3), a convenient tool to monitor autophagic flux based on the different pH stabilities of the EGFP and mRFP fluorescent proteins. The red signals (*i.e.*, green fluorescence-quenched vesicles) appeared in wild-type MEFs within 3 h, whereas red signals in *Prip*-KO cells were infrequently observed in both experiments using *S. typhimurium* and *S. aureus*. To confirm the existence of autolysosome-like acidic vacuoles, we then analyzed the acidification of *S. aureus*-containing autophagosome-like vesicles (SAcVs) in MEFs using LysoTracker blue DND-22, a probe for acidic compartments. LC3-positive SAcVs stained with LysoTracker were observed 3 h post-infection in wild-type MEFs, but there were fewer apparent in *Prip*-KO MEFs.

PRIP is a novel modulator of autophagy

We show here that PRIP is involved in the entrapment of pathogens by LC3-positive autophagosome-like vacuoles and contributes to the autophagic clearance of bacterial pathogens as part of an innate defense system in eukaryotic non-professional phagocytic cells. Following *S. typhimurium* invasion, most bacteria reside and replicate within intracellular compartments termed *Salmonella*-containing vacuoles (SCVs). However, a significant fraction of *S. typhimurium*, which colocalizes with p62 that binds both polyubiquitinated proteins and LC3, is targeted by autophagy, and thus autophagy restricts *Salmonella* replication (Zheng et al., 2009). Phosphatidylinositol 3-phosphate [PI(3)P], and double FYVE domain-containing protein 1 (DFCP1) and WD repeat domain phosphoinositide-interacting proteins (WIPI), binding proteins of PI(3)P, mediates autophagic elimination of intracellular bacteria (Huang et al., 2011; Mauthe et al. 2012; Mostowy et al., 2014). In *Prip*-KO cells, *S. typhimurium* and *S. aureus* can escape the host defense system via autophagy due to the autophagosome/lysosome fusion process being disabled. A possible mechanism in *Prip*-mediated autophagic elimination of bacteria is shown in Fig. 1. The PRIP-mediated elimination of bacteria from infected host cells may give us new insight into potentially effective treatments for infectious diseases.

AKNOWLEDGMENTS

K.H. and T.K. conceived and designed the experiments. K.H. chiefly performed the experiments. This work was supported by the Funding Program for Next Generation World-Leading Researchers [LS087 to TK], and by grants from JSPS KAKENHI [Grant numbers 25861757 and 15K11039 to K.H.]. The authors have no competing interests to declare.

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Exploring the Host Mechanisms that Impact RNA Virus Replication in the Cell Nucleus

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Key words: RNA viruses, Bornavirus, persistent infection, cell nucleus, innate immune responses

ABSTRACT

Bornavirus, a nonsegmented, negative-strand RNA virus, is characterized by highly neurotropic and noncytopathic infection and has several unique features among animal-derived RNA viruses. The most striking feature of this virus is that they readily establish a long-lasting persistent infection in the cell nucleus without causing apparent cell damage. This characteristic makes bornavirus the only animal RNA virus capable of intranuclear parasitism. Therefore, study of this virus allows us to uncover previously unknown interactions between RNA virus and host cells. Recently, we demonstrated that ribonucleoprotein of a mammalian bornavirus, Borna disease virus (BDV), interacts directly with the host chromosome throughout cell cycle and that a host chromatin-binding protein, HMGB1, influences BDV replication and persistent infection. To investigate the roles of HMGB1 in BDV replication, we also identified HMGB1-binding proteins (HBP) in the nucleus of BDV-infected cells. We demonstrated that HBP-1 may play a role in the intranuclear sensing of viral RNP, leading to the repression of BDV replication. In this talk, I will present recent our data on the molecular mechanisms of the persistent infection of BDV and the RNA sensing of host cells in the nucleus, providing novel insights into the interaction between RNA viruses and the host cells.

INTRODUCTION

The cell nucleus is a dynamic organelle that may be considered an inconvenient site for virus infection. Dissolution of the nuclear membrane during mitosis and nucleocytoplasmic trafficking through nuclear pores could lead to the loss or dilution of viral genomes from the nucleus. Alteration of the intranuclear environment by gene expression may also affect virus stability. For persistent DNA viruses, such as gammaherpesviruses [Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus] and papillomaviruses, association of a low copy number of viral episomal DNA with cellular chromosomes ensures their stable retention over long periods of time in such a dynamic environment[1-5]. Residence in the nucleus, however, would not be an easy task for other types of viruses, in particular viruses using RNA as the template for replication.

Among vertebrate RNA viruses, except for the reverse-transcribing retrovirus, only a few families, *Bornaviridae*, *Nyamiviridae* and *Orthomyxoviridae*, enter the nucleus for replication and transcription. However, there is a fundamental difference between these viruses. Bornaviruses show a non-cytopathic replication and readily establish a long-lasting, persistent infection in the nuclei of cultured cells and various tissues[6,7], whereas infections by the viruses of the *Nyamiviridae* and *Orthomyxoviridae*, seem to induce cytopathic effects. These features indicate that bornaviruses are the only known RNA virus that can survive for a long-time within the nuclear membrane. However, the mechanism of how bornaviruses can achieve such a stable intranuclear infection has not been fully understood[8,9].

During viral infection, invading foreign DNA and RNA potently activate the innate and subsequent adaptive immune responses[10,11]. Nucleic acid sensors play critical roles in the induction of immune responses, such as the induction of interferon (IFN), proinflammatory cytokines and chemokines. Toll-like receptor (TLR) 3, TLR7, RIG-I, and MDA5 are best known as RNA sensors in the cytoplasm[10-12]. In addition, DNA sensors, such as TLR9, DNA-dependent activator of IRFs (DAI), IFN-inducible protein 16 (IFI16), and absent in melanoma 2 (AIM2) are known to induce innate and adaptive immune responses[13-16]. It has been believed that the cytoplasm is the center of nucleic acid sensing, because all of these nucleic acid sensors are mainly located in the cytoplasm. On the other hand, IFI16 has been recently shown to act as a nuclear pathogenic DNA sensor in response to KSHV infection[17]. It has been still unclear, however, whether the host cell nucleus has the machineries for sensing the invading viral RNAs.

In this report, with a unique model of intranuclear infection, bornaviruses, we provide new insights into the molecular mechanisms of how the host nucleus stands against invading RNA molecules and how a particular RNA virus can evade such host sensing mechanism in the nucleus.

Bornaviruses

Bornaviruses, enveloped, non-segmented, negative-strand RNA viruses, are the member of the *Bornaviridae* family within the order *Mononegavirales*[7]. A mammalian bornavirus, Borna disease virus (BDV) was identified as the etiological agent of Borna disease, a progressive, non-purulent encephalomyelitis in horses and sheep, in the early 20th century, and was long considered to be the only species in the genus Bornavirus. BDV has been reported to infect a wide range of mammalian species, including human. BDV infection was suggested to be involved in human psychiatric disorders in 1985[18,19], but several more recent reports have challenged this idea[20]. Although sporadic BDV infections are observed in several animals, the natural reservoir of BDV has not yet been identified. The bicoloured white-toothed shrew (*Crocidura leucodon*) is a putative natural host of BDV in central Europe[21,22]. However, in view of the range of its habitat, this does not explain the natural reservoir of BDV in other regions and suggests that there must be other reservoirs[23].

Interestingly, the nucleotide sequences of BDV isolates show extremely high conservation among BDV strains, independent of host species, and region and year of isolation[24]. The lack of species diversity in the genus had long remained an enigma. In 2008, however, viruses that are clearly related to, but distinct from, BDV were identified in psittacine birds suffering from proventricular dilatation disease[25,26]. After this discovery, our knowledge regarding bornaviruses has been rapidly growing. At present, at least six different species within the genus Bornavirus have been found in many vertebrates, including mammals, birds and reptiles[27,28]. Furthermore, very recently, a distinct mammalian bornavirus has been detected in the specimens of three patients, who died with fatal encephalitis with similar clinical signs between 2011 and 2013[29]. Interestingly, all these patients were breeders of variegated squirrels (*Sciurus variegatoides*) and were considered to be infected with the squirrel bornavirus from the breeding animals[29], suggesting that bornavirus is the zoonotic agent having a potential to induce severe symptoms in humans.

The genome of bornavirus is an 8.9 kb negative-strand RNA, which encodes at least six genes: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large protein (L), and one non-structural protein, X. Among these structural proteins, N, P, and L are essential for viral replication and transcription[7]. BDV genomic RNA is packaged into ribonucleoprotein (RNP) complexes, which also contain N and viral RNA-dependent RNA polymerase (RdRp) complex. The RdRp complex consists of P and L and is responsible for replication and transcription of the viral genome. X is a non-structural protein with strong inhibitory activity against BDV RdRp. M plays a critical role in virus particle assembly and budding[30]. BDV enters its target cells using the surface G protein. BDV tropism may be determined by the interaction of G with its receptor, but at present, the cellular receptor responsible for the entry of BDV particles has not yet identified[31].

Mechanism of intranuclear persistent infection of bornavirus

A distinguishing feature of BDV is that it replicates noncytopathically and readily establishes persistent infection in the nucleus of infected cells, indicating that BDV is a highly nuclear-associated RNA virus. Therefore, BDV infection provides a good model to investigate the interaction between the host cell nucleus and invading RNA molecules. To understand how BDV can maintain a persistent infection without the dilution of RNA genome in the nucleus, we examined the intranuclear dynamics of BDV RNPs in infected cells. We found that BDV N and P colocalize in subnuclear dots in the interphase nuclei. The larger dots appeared to be the ring-shaped structure and contained both sense and antisense viral RNAs. We designated the dot structures as vSPOT (viral speckle of transcripts) (Fig. 1A)[9]. These observations suggested that BDV RNP exists in the nucleus by assembling the spheroidal center for replication. To understand the subnuclear distribution of BDV RNP in more detail, we also used the correlative fluorescence microscopy-electron microscopy technique. By using this method, vSPOT appeared to be observed as a high electron-dense, spherical aggregate without membrane and to contain RNP-like structures, which associates with perichromatin fibrils. Altogether, BDV RNPs appear to survive in the interphase nucleus by constructing vSPOT, which may be constructed by using chromatin as a scaffold[9].

We also investigated the dynamics of BDV RNP during the mitotic phases. Intriguingly, at the beginning of the mitosis vSPOT disappeared and the RNP components were diffusely dispersed on the condensed metaphase chromosomes. Furthermore, the viral RNPs appeared to be broadly tethered to anaphase and telophase chromosomes and segregated to daughter cell nuclei along with the mitotic chromosomes (Fig. 1B). These observations suggested that BDV RNP attains a secure entering into daughter cell nuclei and an intranuclear distribution throughout the cell cycle by behaving as a part of mitotic chromosome[9].

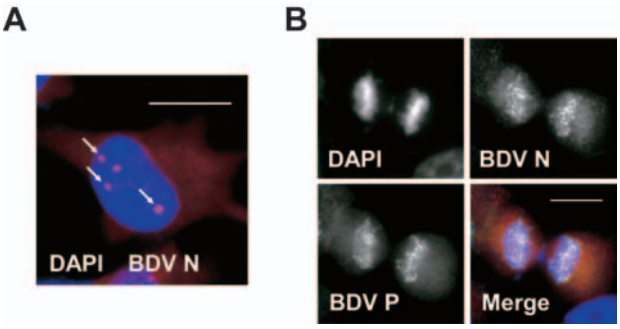

Fig. 1. Intranuclear dynamics of BDV RNPs in infected cells. (A) Confocal microscopic analysis of an interphase nucleus of BDV-infected human oligodendroglioma (OL) cells. BDV generates vSPOTs (arrows) in the nucleus. Cells were visualized by anti-BDV N (red) antibody and DAPI (blue). (B) The telophase chromosomes of BDV-infected OL cells were stained by anti-BDV N (red), anti-BDV P (green) and DAPI (blue) and analyzed by confocal microscopy. Scale bars: 10 μm.

We have previously demonstrated that the chromatin-remodeling, DNA-binding protein, HMGB1 (high mobility group box protein 1) binds to BDV RNPs[32]. We also found that HMGB1 is involved in the stability and transcription of BDV on chromatin by increasing the stability of the P with chromatin-bound RNPs[9], suggesting that bornavirus establishes a life cycle highly associated with the cellular chromosome and takes advantage of chromosomal stability and dynamics to ensure the integrity of its RNP in the infected nucleus.

BDV can establish a long-lasting persistent infection in the nucleus and induce sustained chemokine synthesis in the absence of IFNs during its persistent infection[33], indicating that the study of BDV may allow us to uncover intranuclear machineries for sensing pathogenic RNAs. Intriguingly, HMGB1 is also reported to function as a universal sensor for nucleic acids, triggering the innate immune responses[34]. The binding of nucleic acids to HMGB1 is a precondition for the more efficient, subsequent recognition by discriminative sensors[34]. Therefore, it is conceivable that BDV RNPs may be recognized by HMGB1 and unidentified discriminative sensor (s) in the nucleus by the host to induce innate immune responses. To evaluate this possibility, we now investigating the intranuclear sensing mechanisms for non-self RNAs by using BDV as an infection model of RNA viruses in the nucleus. Our studies could provide a new paradigm of infection of non-reverse transcribing RNA viruses in eukaryotic cells.

ACKNOWLEDGMENT

This study was supported in part by KAKENHI Grant Numbers 26253027, 26670225, 15H01259 (KT), the Core-to-Core Program A, Advanced Research Networks (KT) from the Japan Society for the Promotion of Science (JSPS), Basic Science and Platform Technology Program for Innovative Biological Medicine from the Japan Agency for Medical Research and Development (AMED) (KT).

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Bacteriotherapy for Inflammatory Bowel Diseases

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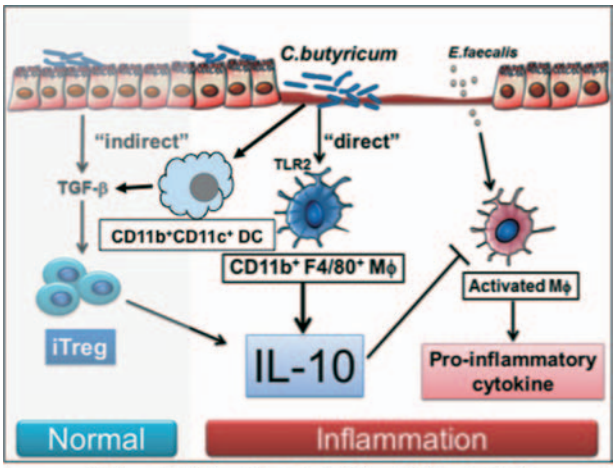
Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic and relapsing inflammatory disorder in the gut. Both the incidence and prevalence of IBD are rapidly increasing, not only in western countries, but also in Japan. In Japan, approximately 160,000 patients with UC and 40,000 with CD are currently registered by Japanese Health, Labour and Welfare Ministry. Genome wide association studies (GWAS) of IBD have implicated genes connected to host interaction with microbiota in their pathogenesis, such as NOD2, CARD9, and ATG16L1, but current rapid increased incidence of IBD cannot be explained by only those genetic backgrounds. Indeed, modern biologic agents targeting the immune system have been effective in patients with IBD, but cessation of treatment leads to relapse in almost all patients, suggesting that intrinsic immune dysregulation is an effect, not a cause, of IBD. The most accepted hypothesis of IBD pathogenesis to date is that the aberrant immune response against the gut microbiota is triggered by environmental factors in a genetically susceptible host. In humans the gastrointestinal tract is home to approximately 10^{14} commensal bacteria of over many thousands of individual strains [2]. The number of bacterial cells exists in the gut showed from 10^{1} to 10^{3} bacteria per gram of contents in the stomach and duodenum, progressing to 10^{4} to 10^{7} bacteria per gram in the small intestine, and rising to an estimated 10^{11} to 10^{12} bacteria per gram in the large intestine. 16S ribosomal RNA gene (rRNA) sequence-based methods have shown that human gut microbiota is dominated only two bacterial divisions, the Bacteroidetes and the Firmicutes phyla, constitute over 90% of the known phylogenetic categories and dominate the distal gut microbiota. The gut microbiota plays a key role in health and disease in humans. Many studies have revealed that an imbalance in the bacterial composition (dysbiosis) provokes host pro-inflammatory immune responses and induces inflammatory diseases. The recent advance of the next-generation sequencing technology enabled identification of dysregulated alterations of the composition of the gut microbiota (dysbiosis) in IBD. Recent progression on gnotobiotic technology and metagenomics have enabled analysis of the role of specific bacterial strains in immunological responses. By taking advantage of these techniques, a recent study showed that the 17 strains of Clostridia belongs to clusters IV, XIVa and XVIII derived from the healthy human microbiota induced accumulation of IL-10-producing induced Treg (iTreg) cells, which suppressed colitis in a dextran sodium sulfate (DSS) colitis model. This emerging evidence shows that specific commensal bacteria can affect the differentiation of mucosal Treg cells under normal conditions, it has remained largely unclear which specific bacteria influence mucosal-specific antigen-presenting cell phenotypes such as macrophages and dendritic cells. Unlike these commensal Clostridium strains, the probiotic Clostridium strain *Clostridium butyricum* (*CB*), which belongs to cluster I primarily induced IL-10 from colonic $F4/80^{+}$ $CD11b^{+}$ intestinal macrophages rather than $Foxp3^{+}$ Treg cells, and prevented experimental colitis by an IL-10-dependent mechanism. Macrophages are essential for maintaining intestinal homeostasis and involving the pathology of IBD. I discuss the role of Treg cells and macrophages in gut homeostasis and inflammation, and describe how the gut microbiota, especially Clostridia, influence the immune system and colitis (Figure).



① Hayashi A, Kanai T, et al. Cell Host & Microbe 2013.
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In addition to a single strain of probiotics, unique therapy correcting dysbiosis by fecal microbiota transplantation (FMT) may be promising in IBD. The first therapeutic use of FMT was reported in 1958 for pseudomembranous colitis. Since then, more than 100 case reports of therapeutic FMT have been published for various diseases; however, until recently, there had been no controlled studies of FMT. In 2013, the first randomized controlled clinical trial of FMT for recurrent CDI was published in New England Journal of Medicine. FMT has finally entered the era of evidence-based medicine. FMT has emerged as a treatment in conjunction with rapid progress in our understanding of the role of intestinal microbiota in health as well as disease. FMT is considered effective in remedying imbalances of the intestinal microbiota. Consequently, FMT can be applied to a variety of diseases in which the intestinal microbiota is disturbed. This category of disease includes not only gastrointestinal disorders, but other systemic disorders

such as metabolic syndrome, diabetes mellitus, non-alcoholic steatohepatitis, autoimmune diseases, and cardiovascular diseases. Although FMT has huge potential in the treatment of various diseases, there remain many unanswered questions on FMT regarding proper indications, optimal protocols, and patient selection, and more research is required. I here introduce such new therapeutic approach for the cure of IBD.

Role of Dental Science for the Development of Mucosal Immunology and Vaccine

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Key words: IgA, M cell, MucoRice, Oral Vaccine, Peyer's patch

ABSTRACT

The aero-digestive tract, which is covered by mucosal epithelium is begun from oral cavity and continuously exposed to outside environment. Since mucosal surface is under the threat of invasion of pathogens, it is thus equipped with the mucosal immune system. In the early period of our scientific endeavors for showing the presence of mucosal immune system, dental science has made significant contribution for their foreseeability to use oral immunity to control oral diseases (e.g., dental caries). The mucosal immune system is separated functionally into, but interconnected inductive and effector sites. For the digestive tract, Peyer's patches (PPs) are a well characterized inductive site containing antigen-sampling M cells and immunocompetent cells required for the initiation of antigen-specific immune responses. In the effector sites (e.g., intestinal lamina propria and salivary gland), PP-originated antigen-specific IgA B cells become plasma cells and produce polymeric IgA and form secretory IgA (SIgA) by binding to poly-Ig receptor expressed on epithelial cells for the dual effects of protective and harmonized immunity against pathogenic and commensal microorganisms, respectively. For the induction of protective immunity, oral vaccination has been shown to be an attractive immunization route for the induction of antigen-specific SIgA response. The development of new-generation mucosal vaccines, including the rice-based oral vaccine "MucoRice", on the basis of the coordinated mucosal immune system fused with plant transgenic technology is a promising strategy for the control of mucosal infectious diseases.

ABBREVIATIONS

CCL, CC chemokine ligand; CCR, CC chemokine receptor; CT, cholera toxin; CTB, CT B subunit; GALT, gut-associated lymphoid tissue; IgA^+ B cells, IgA-committed B cells; LT, heat-labile enterotoxin; MALT, mucosa-associated lymphoid tissue; M cell, microfold or membranous cell; PP, Peyer's patch; SIgA, secretory IgA; TGF-β, transforming growth factor β; Th, helper T; VHH, variable domain of llama heavy-chain antibody fragment

1. Introduction

Beginning of oral cavity, aero-digestive tract covers the largest surface area of the human body, and it forms the boundary between the interior of the body and its external environment[1]. Because of its large surface area and continuous exposure to the outside environment, the aero-digestive mucosa is the primary invasion site of most pathogens via inhalation and ingestion[2]. Therefore, the host is equipped with a dynamic and flexible immunologic barrier, mucosal immune system for providing appropriate protection against invasion by harmful pathogens. At same time, the aero-digestive mucosa has essential roles in the physiologic functions of inhalation and ingestion and therefore is integral to these basic life-supporting systems. These concurrent yet contrasting roles—immunologic barrier and physiologic machinery—are a unique characteristic of the aero-digestive mucosa.

In order to provide an appropriate mucosal barrier, the innate and acquired immune systems must be cooperatively carried out. The mucosal immune system is thus equipped with physical barriers—consisting of tight junctions and a dense layer of mucins—and biochemical barriers—provided by antimicrobial peptides— and innate immunity, including Toll-like receptors and innate immune cells (e.g., natural killer cells, natural killer T cells, innate lymphoid cells mast cells, and eosinophils)[2]. For acquired immunity at the aero-digestive mucosal epithelium, antigen-specific secretory IgA (SIgA) immune responses are essential players in preventing pathogen invasion. SIgA can neutralize the infectivity of pathogens[3,4] and contributes to the creation and maintenance of mucosal homeostasis[5].

2. Induction of Antigen-specific SIgA Responses

For generating antigen-specific SIgA antibodies, antigens have to be presented directly to the mucosal surface—especially antigen-sampling M cells and antigen-presenting cells (e.g., dendritic cells) located in the mucosa-associated lymphoid tissue (MALT)[2,6,7]. Therefore, oral delivery of antigens that target Peyer's patches an example of gut-associated lymphoid tissue (GALT) leads to the generation of antigen-specific SIgA

antibodies in the secretions of the oral cavity and intestine[6]. In contrast, although very effective in the induction of systemic immunity (e.g., serum IgG antibody), the injection of antigen, apparently does not induce antigen-specific SIgA antibody responses[7,8]. These facts indicate that new-generation vaccines must be rationally designed to efficiently induce antigen-specific mucosal immunity at the entry sites of mucosal pathogens.

The mucosal immune system consists of coordinated inductive and effector tissues (Fig. 1). As inductive sites, MALTs are furnished with all of the necessary antigen-sampling presenting and immunocompetent cells for the initiation of antigen-specific immune responses[6-8]. For example, at Peyer's patches (PPs), orally administered antigens are taken up by M cells located in the follicle-associated epithelium. M cells then deliver antigens to antigen-presenting cells, such as dendritic cells and macrophages, that lie beneath the follicle-associated epithelium of PPs for the subsequent presentation of processed antigen leading to the generation of Th1 cells, Th2 cells, Th17 cells, follicular helper T cells or cytotoxic T cells and the production of IgA-committed B cells (IgA^+ B cells)[6]. Concurrent with antigen presentation, dendritic cells located in PPs induce gut-imprinting molecules (e.g., CC chemokine receptor [CCR]9, CCR10, α4β7 integrin) on antigen-specific lymphocytes through the retinoic acid cascade for their subsequent migration to the effector tissues (e.g., intestinal lamina propria)[7,8] (Fig. 1). The lipid mediator system, which includes sphingosine-1-phosphate and its receptor, sphingosine-1-phosphate receptor type 1, plays a critical role in the egress from PPs of antigen-specific lymphocytes that carry gut-imprinting molecules and in their subsequent immunologic journey to distant effector sites[9,10].

At the effector sites such as salivary gland and intestine, PP-originated antigen-specific Th2 cells provide the IgA-enhancing cytokines (including IL-5, IL-6, and IL-10) required for the final differentiation of IgA^+ B cells into plasma cells that produce dimeric or polymeric forms of IgA[6,11,12]. These IgA antibodies then bind to poly-Ig receptors expressed on the basal membrane of epithelial cells, where they form SIgA and are transported to secretions[13,14] (Fig. 1). This interconnected and well-orchestrated process between the inductive (e.g., PPs) and effector (e.g., oral cavity and intestine) sites provides the immunologic basis for the induction and regulation of antigen-specific immune responses (e.g., SIgA production) at the mucosal surface[2,6-8].

3. Dental Science Contributed for the Creation of Mucosal Immunology

In past several years, mucosal immunology has

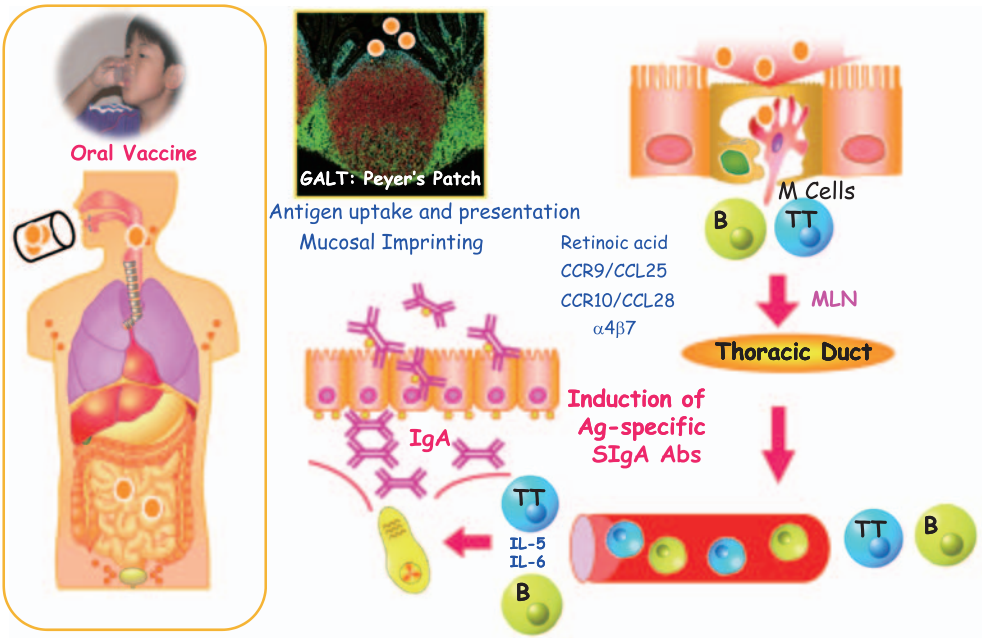

Fig. 1. The Mucosal immune system for the induction and regulation of antigen-specific immune responses
Orally delivered antigens in the lumens of the gastrointestinal tract are endocytosed by M cells located on the follicle-associated epithelium (FAE) of gut-associated lymphoid tissue (GALT), or Peyer's patches, M cells located in the FAE form the subepithelial dome structure, and antigen-presenting cells (APCs) such as dendritic cells (DCs) lie immediately beneath the FAE. M-cell-endocytosed antigens are immediately processed by DCs, which transport antigens to underlying T and B cell zones for priming of antigen-specific T cells and IgA-committed B cells (IgA^+ B cells). These antigen-specific antigen-specific lymphocytes acquire mucosal-imprinting molecules, such as CCR9, CCR10, and α4β7 integrin, and subsequently migrate to the effector sites. At the effector sites (e.g., salivary gland and intestinal lamina propria), IgA^+ B cells differentiate into plasma cells after stimulation by the IgA-enhancing cytokines IL-5, IL-6, and IL-10, which are secreted by antigen-specific Th2 cells. Dimeric or polymeric IgA secreted from plasma cells is transported to the mucosal surface as secretory IgA (SIgA) through the binding to polymeric Ig receptor expressed on the basal membrane of epithelial cells.

turned into a core entity of immunology, microbiology, allergology and pathology. Before the 1970s, limited numbers of immunologist recognized the presence of the immune system at the mucosal surface of the aero-digestive tract. A major scientific contribution during that uncredited era was initiated by the demonstration for the presence of IgA antibodies in saliva[15,16]. It was shown that human parotid saliva (and other nonvascular fluids) contained large amounts of IgA and these IgA antibodies showed chemically and immunologically unique properties when compared with serum IgA[15-17]. Several investigators, including those in our group, with backgrounds in dentistry and oral biology were aware of the prominent relationship between the oral cavity as the beginning of the digestive tract and the presence of large quantities of IgA antibodies (~200 mg) in the saliva (~750-1000 mL) and swallowed each day[18]. It is interesting to note that although *Streptococcus mutans* was first isolated in 1924, most of the research formally proving its role as the causative pathogen in dental caries occurred in the 1960s and 1970s[19,20]. These immunological and microbiological advances in the dentistry yielded the scientific strategy for developing a caries vaccine that induced the production of *S. mutans*-specific SIgA in the salivary glands and of gingival fluid IgG produced in the serum[21,22]. Our earlier study demonstrated that oral or nasal administration of *S. mutans* resulted in the induction of both antigen-specific salivary IgA and serum IgG antibodies[23,24]. Scientific efforts made by several investigators in the fields of dental science and oral biology became a compelling force behind the wider scientific community's current acceptance of the mucosal immune system and of oral immunization as an effective way to induce antigen-specific SIgA production in mucosal secretions.

4. Development of New-Generation of Rice-based Oral Vaccines

Currently, most of the vaccines clinically available for human use are given through systemic routes (e.g., injection). The injection type of vaccination is effective in the induction of protective immunity in the systemic compartment, but in general elicits very weak, or no, antigen-specific immune responses at mucosal surfaces, which are major invasion sites of pathogen[7,8]. Although the injection is currently and routinely used for vaccination to induce protective immunity within the body, it may not be appropriate for providing suitable protection against the invasion of harmful mucosal pathogens. On the other hand, oral vaccination with appropriate delivery vehicles or co-administered with adjuvant has successfully induced not only systemic but also mucosal immune responses, leading to a double layer of protec-

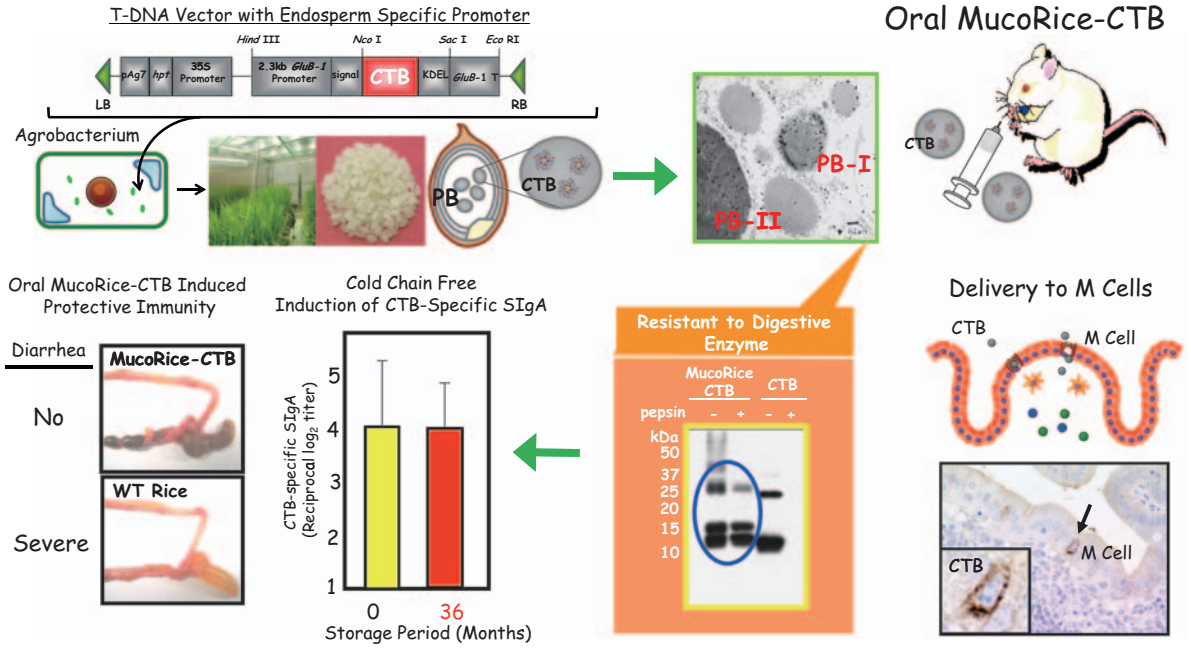

Fig. 2. Rice-based oral vaccine MucoRice
The gene encoding cholera toxin B subunit (CTB) is transduced into rice seed by means of an *Agrobacterium*-mediated method. The resultant recombinant CTB protein (rCTB) accumulates in the rice protein body (PB), which is a unique protein-storage organelle.
When the section of MucoRice-CTB seeds was stained with gold particle- conjugated anti-CTB, electron microscopic analysis showed that MucoRice-CTB preferentially expressed CTB in the protein body (PB)-I and -II of rice seeds. MucoRice-CTB were treated with or without pepsin (0.5 mg/ml) for 1hr at 37°C and then subjected to Western blotting analysis. The purified rCTB protein was easily digested by pepsin, whereas the CTB protein expressed in the protein body of rice (MucoRice-CTB) was resistant to digestive enzymes. In addition, MucoRice-CTB was stable at room temperature for years and thus the amount of CTB expressed in the rice seeds did not change after 18 months storage period. Following oral immunization, MucoRice-CTB is delivered effectively to gut mucosal surfaces and is endocytosed by M cells located follicle associated epithelium of Peyer's patch. Oral administration of MucoRice-CTB in mice effectively elicited CT-neutralizing serum IgG and fecal IgA antibodies and protects mice from the diarrhea induced by orally challenged CT. Some of data are adopted from references 30 and 38.

tion against mucosal pathogens[25]. Further, mucosal vaccines provide other advantages over injectable vaccines including increased ease of administration, less physical and psychologic discomfort, and avoidance of needlestick injuries and transmission of blood-borne diseases[7,8].

In spite of these noticeable advantages, only a few mucosal vaccines have been approved for clinical use in humans: vaccines against poliovirus, rotavirus, *Salmonella typhi*, and *Vibrio cholerae* are administered orally, and vaccines against influenza virus are given intranasally[8]. Most of these currently available oral and nasal vaccines involve either attenuated or gene-modified live or killed forms of whole microorganisms[8]. A mucosal vaccine that delivers a component (subunit) or purified form is not yet available for clinical use. Oral immunization of a protein based vaccine antigen alone fails to effectively induce antigen-specific immune responses due to intrinsic physiologic mechanisms of the digestive tract, namely degradation of vaccine antigen by digestive enzymes (e.g., pepsin[26]); clearance mechanisms (e.g., peristalsis action and mucus secretion); and physiologic and biologic barriers (e.g., gastric acids, mucins, and tight junctions) to the access of vaccine antigen to intestinal inductive tissue (e.g., Peyer's patches)[6,8]. Although various obstacles remain, an oral vaccination strategy appears attractive and beneficial for the next generation of vaccines. Furthermore, recent scientific advances achieved through the sharing of knowledge and technologies among different fields of science are helping to overcome various hurdles in the development of oral vaccines.

For the development of oral vaccine, transgenic plant-based vaccines are spotlighted because of their practicality, safety, and low cost[7,27-29]. When compared with injectable vaccines, oral vaccines require additional technological advances in antigen-delivery techniques, because the antigens must cross intestinal barriers comprising digestive enzymes, mucus, and physiologic mechanisms to be presented to gut inductive site (e.g., Peyer's patches). Among the diffrent plant candidates (e.g., carrot, potato, rice, soybean, tobacco, and tomato) for transgenic vaccines composed of a bacterial component of heat-labile enterotoxin [LT] B subunit, cholera toxin B subunit [CTB], or *Yersinia pestis* and a viral component of hepatitis B virus, rotavirus, or norovirus[30-37], rice may be the most suitable antigen-expressing plant because rice seed (especially the protein body) is resistant to digestion by gastric acid[8,27,30]. Furthermore, protein expressed in rice seeds is stable for a prolonged time in the absence of refrigerated storage[30]. Rice can thus be considered a viable candidate for the creation of cold-chain-free and needle-free vaccine.

MucoRice-CTB, which consists of CTB expressed in transgenic rice, is one of the most potent plant-based vaccines (Figure 2)[30]. The CTB gene was inserted into rice seed by use of an *Agrobacterium*-transformation system (Figure 2) which resulted in the production of recombinant CTB protein reaches 15-30 μg per transgenic rice seed[30]. These CTB proteins are accumulated in the rice protein body, a unique protein-storage organelle in rice seed (Figure 2)[30]. The CTB proteins expressed in the protein body are stable at room temperature for more than 3 years without loss of immunogenicity and also resistant to intestinal digestive enzymes (Figure 2)[30,38]. These results suggest that the rice protein bodies are acting as a natural capsule for the oral delivery of vaccine antigens to the gut immune system. Thus, oral delivery of MucoRice-CTB leads to its subsequent uptake by M cells and transport to the inside of Peyer's patches (Figure 2)[8,27].

In addition to its advantages of refrigeration-free storage and effective oral delivery, MucoRice-CTB effectively induces antigen-specific protective immune responses in both the systemic and mucosal compartments (Figure 2). Oral vaccination of MucoRice-CTB in mice induces CT-neutralizing serum IgG and fecal IgA antibodies and protects mice from diarrhea induced by orally challenged CT (Figure 2)[30,38]. MucoRice-CTB also induces CT-specific neutralizing antibody production in non-human primates[39]. Orally administered MucoRice-CTB induces cross-protective immunity against LT[38]. The cross-protective immunity against LT induced through vaccination with MucoRice-CTB is thought to reflect the close similarity between the CTB and LTB subunits[38,40]. Therefore, MucoRice-CTB may be a potential vaccine against the toxin produced by enterotoxic *E. coli*, which is a major cause of severe diarrhea in children in developing countries[38,41].

The MucoRice system is also applicable to oral passive immunotherapy because of its advantages of both stability in the absence of refrigeration and effective oral delivery[42]. MucoRice-ARP1 expresses the anti-rotavirus specific variable domain of llama heavy-chain antibody fragment (VHH) in rice seeds and is a candidate for a new oral passive immunotherapy against rotavirus infection[42]. VHH, which is composed of heavy-chain dimers and is devoid of a light chain, possesses an extensive antigen-binding repertoire[43], and because rotavirus-specific VHH (ARP1) has been efficiently produced in yeast and reduces morbidity from rotaviral diarrhea in mice[44], its application to passive immunotherapy for rotaviral infection offers an attractive strategy for the control of rotavirus infection, in addition to the currently available rotavirus oral vaccines. MucoRice-ARP1 comprises the 12-kDa ARP1 protein expressed in transgenic rice (0.85% of seed weight) and has all the advantages described for MucoRice-CTB, such as stability at room temperature[42]. Further, oral administration of MucoRice-ARP1 attenuates rotaviral infection in both immunocompetent and immunodeficient mice, suggesting that MucoRice-ARP1 can be used for the immunocompromised population in addition to healthy subjects. The combined technologies of the MucoRice expression system and VHH are expected to evolve into oral passive immunotherapy options for diverse mucosal infectious diseases[42].

5. Conclusion

The mucosal surface of aero-digestive tract harbors a dynamic and flexible immune network that protects the host against external pathogens. The digestive tract begin with oral cavity has its own immune inductive tissue—the so-called GALT including Peyer's patches. The inductive tissue possess unique functional characteristics, including antigen sampling by M cells and subsequent

processing and presentation by antigen-presenting cells, resulting in the initiation of antigen-specific mucosal immune responses. Th1, Th2, Th17, and follicular helper T cells and cytotoxic T cells, as well as IgA^+ B cells, are thus generated in PPs and then migrate to the distant intestine and oral cavity to provide the first line of defense (e.g., SIgA).

The importance of mucosal immunity is well known, however, traditional systemic vaccines, which typically are administered by injection, fail to induce antigen-specific mucosal immune responses and thus cannot provide protection against the invasion of pathogens at epithelial surfaces. Despite our increased knowledge of the need to maximize the effective induction of both antigen-specific systemic and mucosal immune responses, few mucosal vaccines are currently available. However, scientific and technological fusions among the fields of mucosal immunology, bioengineering, agricultural science, plant biology and plant factory have led to the creation of new-generation rice-based oral vaccine (e.g., MucoRice). Continued concomitant progress in characterizing the underlying aspects of the mucosal immune system and their application to mucosal vaccines relies not only on the scientific advancement of mucosal immunology but also on novel mergers of modern concepts and technologies from both related and unrelated fields.

ACKNOWLEDGEMENTS

Our studies were supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Scientific Research S); the Global Center of Excellence Program of the Center of Education and Research for Advanced Genome-based Medicine; MEXT Translational Research Network Seed C; and the Core Research for Evolutional Science and Technology Program of the Japan Science and Technology Agency. I thank Dr. T. Azegami for helping the preparation of this review article.

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Session IV

Development and Function of the Musculoskeletal System

The Anti-angiogenic Actions of Tenomodulin, a Specific Marker for Tendons and Ligaments

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Developmental Modularly of Secondary Skeletal Elements Involves a Distinct Population of Progenitor Cells

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The Intervertebral Disc: Working Together in Harmony; Breaking up in Pain

The University of Hong Kong
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The Anti-angiogenic Actions of Tenomodulin, a Specific Marker for Tendons and Ligaments

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ABSTRACT

Tenomodulin (Tnmd) is a type II transmembrane glycoprotein consisting of 317 amino acids in mouse and human. The C-terminal cysteine-rich domain of Tnmd is homologous to that of Chondromodulin-1 (Chm1), an angiogenesis inhibitor purified from epiphyseal cartilage. *Tnmd* is predominantly expressed in hypovascular dense connective tissue including tendons, ligaments, epimysium of skeletal muscle, sclera, and cornea. In tendons and ligaments, Tnmd is specifically localized to the avascular region of tendons negative for CD31, a marker of vascular endothelial cells. The mRNA level of *Tnmd* in tenocytes is significantly upregulated by overexpression of *Scleaxis* (*Scx*) that is a basic helix-loop-helix transcription factor regulating maturation of tendons and ligaments, while Tnmd expression is almost abolished in tendons and ligaments of *Scx* null mice. The C-terminal portion of Tnmd including eight cysteine residues is inhibitory to proliferation, migration, tube formation of vascular endothelial cells as well as angiogenesis-dependent tumor growth. In the heart, the local absence of Tnmd in the cardiac tendineae cordis causes angiogenesis and matrix metalloproteinase activation. Thus, Tnmd is an anti-aniogenic molecule in hypovascular dense connective tissue such as tendons and ligaments.

INTRODUCTION

Most mesenchymal tissues including bones and muscles are highly vascularized, but cartilage is avascular and resistant to neovascularization as well as tumor invasion (Hayami et al., 1999; Shukunami et al., 1999). Similarly, tendons and ligaments have low vascularity (Shukunami et al., 2008). It has been postulated that these avascular or hypovascular mesenchymes may contain some anti-angiogenic molecules. We cloned *Tnmd* as a gene related to *Chm1* that encodes an angiogenesis inhibitor extracted from cartilage (Hiraki et al., 1997; Shukunami et al., 2001). As shown in Figure 1, the C-terminal portion of Chm1 (120 amino acids) is cleaved from the Chm1 precursor (334 amino acids) at its furin-processing signal RERR and is secreted out of chondrocytes (Hiraki et al., 1997; Hiraki et al., 1999), whereas Tnmd (317 amino acids) is expressed on cell surface as a type II transmembrane protein (Shukunami et al., 2001). Both Tnmd and Chm1 have the BRICHOS domain with a pair of conserved cysteine residues (Figure 1). The BRICHOS family of proteins consists of five members including BRI2 related to familial British and Danish dementia, surfactant protein C, CA11 related to stomach cancer as well as Tnmd and Chm1 (Sanchez-Pulido et al., 2002; Shukunami and Hiraki, 2007).

Distribution of Tnmd

Tnmd is expressed in dense connective tissues including tendons, ligament, sclera, epimysium of skeletal muscle, sclera and cornea of the eye, all of which are hypovascular or avascular (Oshima et al., 2004; Shukunami et al., 2001; Sugimoto et al., 2013), whereas *Chm1* is expressed in avascular cartilage, eye, and heart valve (Funaki et al., 2001; Kusafuka et al., 2002; Shukunami et al., 1999). Double immunostaining revealed that both Chm1 and Tnmd are localized to the CD31 negative avascular region of each expression domain (Shukunami et al., 2008). Type I collagen, a major component of extracellular matrix in connective

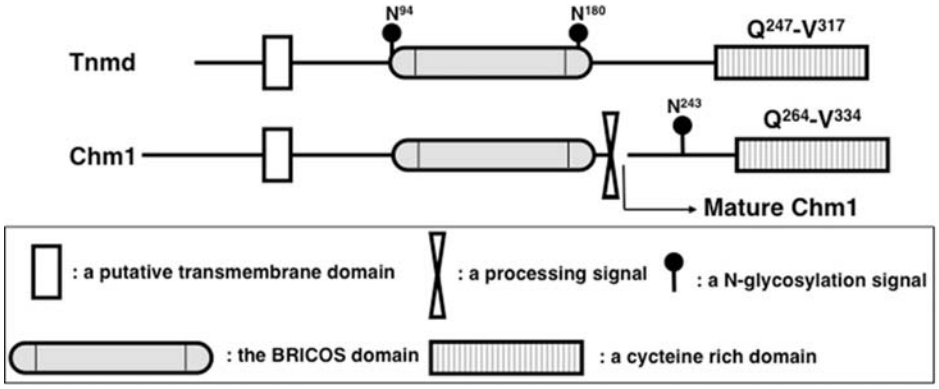

Figure 1. Structural characteristics of Tnmd and Chm1.

tissues, is distributed in dense and loose connective tissues, but Tnmd is only localized to dense connective tissues where thick bundles of aligned collagen fibers are found (Shukunami et al., 2006). In tendons, elongated tenocytes (or tendon fibroblasts) are aligned in parallel to thick mature collagen fibers to form the regular layer, whereas oval tenocytes are randomly distributed in the interlaminar spaces. Tnmd expression is not detected in oval tenocytes but in elongated tenocytes (Shukunami et al., 2006), suggesting that Tnmd is a good marker of mature tenocytes *in vivo*.

Anti-angiogenic action of Tnm

Both Tnmd and Chm1 precursor are type II transmembrane glycoproteins that have the anti-angiogenic domain at the C-terminus. The C-terminal domain has eight cysteine residues to form four disulfide bridges (Miura et al., 2010; Miura et al., 2012; Shukunami et al., 2006) and is well conserved across species (Sachdev et al., 2001; Shukunami et al., 2006). Overexpression of mature Chm1 and the C-terminal portion of Tnmd (116 amino acids) inhibited proliferation, migration, and tube formation of human umbilical vein endothelial cells as well as human retinal vascular endothelial cells (Oshima et al., 2003; Oshima et al., 2004). When malignant melanoma cells overexpressing either of these molecules are inoculated into syngeneic mice, tumor growth was significantly suppressed due to inhibition of angiogenesis (Oshima et al., 2004). In the heart, the local absence of Tnmd in the cardiac tendineae cordis induces angiogenesis and matrix metalloproteinase activation (Kimura et al., 2008), whereas loss of Chm1 caused enhanced vascular endothelial growth factor (VEGF)-A expression, lipid deposition, and mineralization in the cardiac valve in aged mice (Yoshioka et al., 2006).

Regulation of Tnmd expression by Scx

Scx is induced in the early stages of tendon/ligament development, while *Tnmd* expression is more associated with maturation of tendons and ligaments (Shukunami et al., 2006; Yukata et al., 2010). *Tnmd* is coexpressed with *Scx* in mature tendons and ligaments during development and postnatal growth (Shukunami et al., 2006; Komiyama et al., 2013). *Tnmd* expression is upregulated in both tenocytes and periodontal ligament (PDL) cells by Scx (Alberton et al., 2012; Shukunami et al., 2006; Takimoto et al., 2015). Conversely, knockdown of *Scx* in PDL cells downregulated the expression of *Tnmd* (Takimoto et al., 2015). Consistent with these results, Tnmd expression is abolished in hypoplastic tendons and ligaments of *Scx* knockout newborn mice. Thus, Scx is an important regulator for Tnmd during tendon and ligament development.

CONCLUSION

Tnmd is a good cell surface marker for tenocytes and ligamentocytes both *in vivo* and *in vitro*. The cysteine-rich Chm1 like domain of Tnmd has the anti-angiogenic and anti-tumorigenic activities. Scx is indispensable for the expression of Tnmd during development and postnatal growth.

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Developmental Modularly of Secondary Skeletal Elements Involves a Distinct Population of Progenitor Cells

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Key words: Skeletogenesis, Modularity, Progenitor cell, Patterning, Morphogenesis, Bone eminence, Sesamoid bone, *Sox9*, Scleraxis, TGFβ, BMP4

ABSTRACT

Secondary elements such as bone eminences and sesamoid bones are essential for the function of the musculoskeletal system. Nevertheless, their development has been largely understudies. Recently, we reported that in mouse embryos, both bone eminences and the patella, which is the largest sesamoid bone in the human body, originate in a distinct population of progenitor cells. These progenitors uniquely express both *Sox9*, a chondrogenic marker in developing bones, and scleraxis, which is typically expressed by all tendon cells and progenitors. This progenitor pool was also shown to be regulated separately, as TGFβ was necessary for its specification and BMP4 signaling was required for the differentiation of progenitor cells to chondrocytes. These finding reveal a common mechanism for modular development of secondary skeletal elements, which enhances the plasticity in skeletal development and evolution.

INTRODUCTION

Over the past two centuries, the development of the skeletal system has been extensively studied (Lefebvre and Bhattaram; Olsen et al., 2000), resulting in a substantial body of knowledge. Nevertheless, several important aspects of this developmental process have remained largely neglected. Among these is the development of auxiliary elements such as bone eminences and sesamoid bones.

A key step in the assembly of the musculoskeletal system is the attachment of muscles via tendons to bones. Tendons are often inserted into bone eminences, superstructures that protrude from the bone surface and exhibit a variety of shapes and sizes (Gray, 1918; Hill, 1964). Bone eminences provide stable anchoring points for tendons and dissipate stress concentrated at the insertion site, which improves the mechanical resilience of muscle attachment and facilitates movement (Benjamin et al., 2002; Biewener et al., 1996; Thomopoulos, 2011; Thomopoulos et al., 2010). Because of their prominence in the bone landscape, these structures also contribute to the unique three-dimensional morphology of each bone (Gray, 1918).

The current view of skeletal patterning fails to explain the formation of sesamoid bones. Named after their morphological resemblance to the sesame seed, these small and flat bones share the unique property of being superficially embedded within tendons, notably tendons that wrap around joints. The most recognized and studied sesamoid bone is the patella, also known as the kneecap. The patella, which is the largest sesamoid in the human body, is part of the patellofemoral joint, one of two joints composing the knee (Pearson and Davin, 1921a). It has a crucial effect on the mechanics and stability of the knee, increasing the distance between the quadriceps muscle and the knee and thereby the moment arm of the muscle (Schindler and Scott, 2011). Until recently, sesamoids had been thought to develop inside tendons in response to mechanical signals from the attaching muscles. However, this widely accepted model lacked substantiation.

In recent years, we have investigated bone eminence and sesamoid bone development in two separate studies. Our surprising results have uncovered a common developmental strategy, based on modularity and a previously unknown progenitor population.

Bone Eminences Are Formed Modularly by a Distinct Pool of *Scx-Sox9* Double-Positive Progenitors

Several studies have provided a histological and, to some extent, molecular description of the mature tendon-to-bone insertion site, known as the enthesis (Benjamin et al., 2002; Biewener et al., 1996; Thomopoulos et al., 2011; Thomopoulos et al., 2010). To address the fundamental question of how tendons are attached to bones during musculoskeletal assembly, we have focused on the development of the bone eminence. Previously, we showed that the development of these superstructures is mediated by a miniature growth plate, under regulation of signals from tendons cells and, subsequently, of muscle-induced mechanical signals (Blitz, 2009; Seo and Serra, 2007; Spagnoli et al., 2007). Focusing on early patterning events, we recently discovered a unique pool of progenitor cells that give rise to bone eminences (Blitz et al., 2013). Long bones develop by endochondral ossification, during which a cartilaginous template of the future bone is formed first and is then replace by ossified tissue. We show that two distinct pools of progenitors form the car-

tilaginous template in a modular fashion. Whereas progenitors expressing the transcription factor SRY-box 9 (*Sox9*) form the primary, cylindrical structure of the cartilaginous anlage, a previously unknown second pool of *Sox9* and scleraxis (*Scx*) double-positive progenitors is dedicated to the formation of bone eminences. Later in development, the expression of the basic helix-loop-helix (bHLH) transcription factor *Scx* is restricted to forming tendons (Schweitzer et al., 2001). However, previous works reported early and extensive expression of *Scx* in the limb bud mesenchyme (Asou et al., 2002; Cserjesi et al., 1995).

Intriguingly, while our work provides the first indication for developmental modularity in bones, this strategy has previously been reported in the mammalian heart. During development, the heart is formed by cells from two distinct pools, known as the first and second heart fields. Initially, the heart tube is formed by cells of the first field. Then, cells that originate in the second heart field contribute to its elongation and to the formation of the outflow tract (Kelly et al., 2001; Mjaatvedt et al., 2001; Waldo et al., 2001). This similarity highlights the use of modularity in organogenesis in order to allow morphological and functional complexity.

The discovery of a second pool of progenitor cells that form bone eminences suggests that a different regulatory mechanism controls the specification and differentiation of these progenitors. Indeed, by blocking the expression of *Tgf-βRII* in limb mesenchyme we show that TGFβ signaling exclusively regulate the specification of bone eminence progenitors. Previously, tendon cells have been implicated in the initiation of bone eminence formation (Blitz, 2009; Seo and Serra, 2007; Spagnoli et al., 2007). It is tempting to speculate that by controlling both tendon and bone eminence formation, TGFβ signaling is a key regulator of tendon-bone attachment. Indeed, TGFβ was suggested to coordinate cartilage and tendon differentiation during limb development (Lorda-Diez et al., 2009). However, the mechanism that underlies the role of TGFβ signaling in specification of eminence progenitors is still unclear, especially in light of the broad expression of *Tgf-βRII* and TGFβ ligands in the developing limb (Pryce et al., 2009).

Previously, we showed that the SCX-BMP4 pathway induces eminence formation (Blitz, 2009). Subsequently, we showed that this pathway regulates the differentiation of eminence progenitors into chondrocytes. The expression of *Scx* by eminence progenitors raises a new hypothesis that *Bmp4* expression may be regulated by SCX within the attachment unit, thus ruling out a non-autonomous role for tendons. Gene expression analysis revealed an overlap in the expression domains of *Sox9* and *Scx* in the forming attachment unit between E11.5 and 12.5. Yet, by E13.5 the domains were separated, as *Scx* expression became restricted to tendon cells. At that exact stage, SCX drives *Bmp4* expression in cells at the tip of the tendon (Blitz et al., 2009) and *Sox9*-positive cells undergo differentiation to chondrocytes. This suggests that in the forming tendon, SCX drives *Bmp4* expression to regulate non-autonomously the differentiation of *Sox9*-positive cells to *Sox9/Col2a1*-positive chondrocytes, which form the bone eminence. Because TGFβ signaling was previously shown to regulate *Scx* expression (Murchison et al., 2007; Pryce et al., 2009), it is possible that TGFβ and BMP4 signaling together form a hierarchical mechanism that coordinates specification and differentiation of bone eminence progenitors.

On the Development of the Patella

Traditionally, the developmental program of sesamoid bones has been regarded as an exception, since no canonical skeletogenic program could explain their unique property of being separate from the main skeleton and integrated with a tendon. Studying the development of the mouse patella as a model system, we recently showed that similarly to bone eminences, the patella forms by a distinct pool of progenitors expressing both *Sox9* and *Scx* under the regulation of TGFβ and BMP signaling (Eyal et al, 2015). Previously, it was reported that patella cells are descendants of *Scx*-positive cells and that patella development is arrested when *Sox9* expression is blocked in *Scx*-positive cells (Sugimoto et al., 2013). We also showed that the patella progenitor population first constitutes an integral part of the anteriodistal surface of the femur. Later in development, the patella separates from the femur by a process of joint formation, which is mechanically regulated.

Comparative anatomy studies dating back to the early 19th century have resulted in the postulation of two opposing models for the development of sesamoid bones. The first model suggested that sesamoids develop as intratendinous cartilage in response to mechanical stimuli applied to the functional tendons by muscles (Parsons, 1904; Parsons, 1908). On the other hand, the "detachment" model suggested that sesamoids arise from bony processes that are separated from the skeleton through a process that was then unknown (Pearson and Davin, 1921a; Pearson and Davin, 1921b). Biomechanical studies tilted the balance towards the intratendinous model, which has been widely adopted (Hall, 2005). Our finding that the patella initially forms as part of the femur agrees with the theoretical model raised by Pearson. Moreover, cellular and molecular indications for joint formation between the rudimentary patella and femur provide an explanation for the detachment phase. Analysis of paralyzed embryos revealed that muscle contraction was necessary for joint cavitation during the separation of the patella from the femur. Consistently, we previously showed that muscle contraction is necessary for maintenance of joint progenitors and for proper cavitation (Kahn et al., 2009).

A paradigm for plasticity in skeletal development

The findings of our two studies suggest a common developmental mechanism for bone eminences and sesamoid bones such as the patella. The foundations of this mechanism are a distinct pool of progenitors that uniquely express both *Sox9* and *Scx*, development of the element as a separate module that is either added onto the bone shaft or detached from it, and a regulatory mechanism involving TGFβ and BMP signaling. From an evolutionary viewpoint, modular pools of progenitors increase the plasticity in skeletal patterning and morphogenesis. Modularity also provides an efficient mecha-

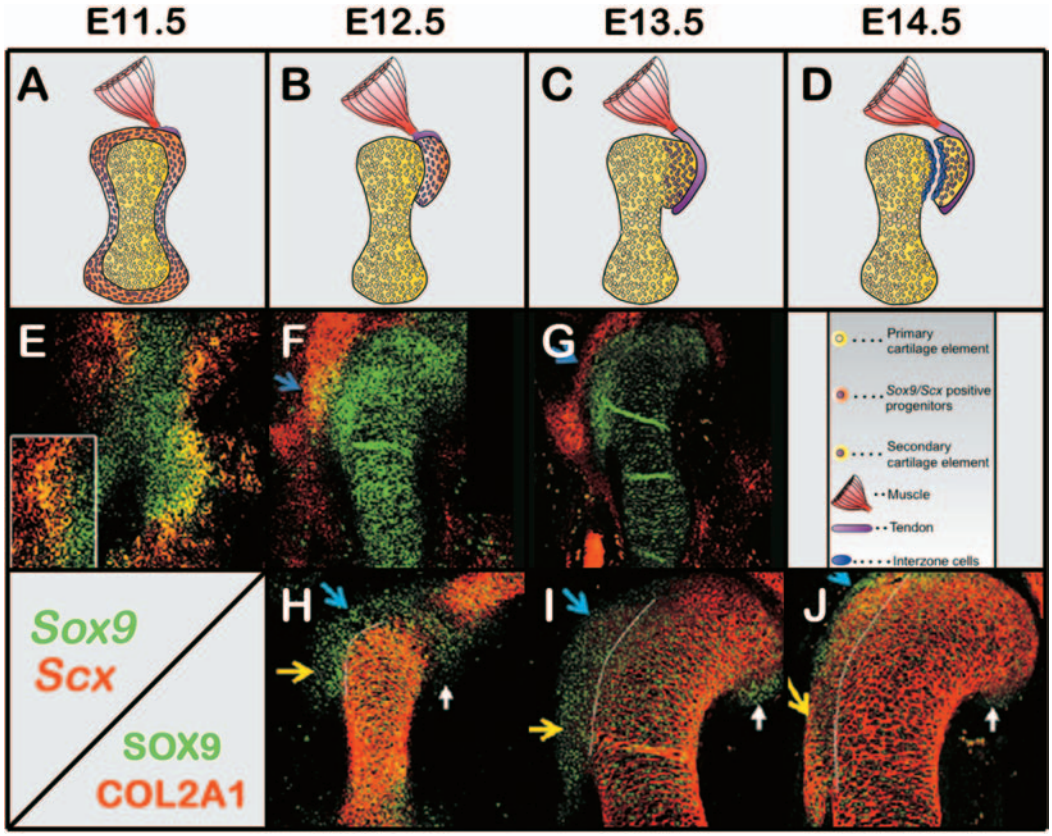

Figure 1. Bone eminences and sesamoid bones share a common developmental strategy. The first phase involves specification of the *Sox9*- and *Scx*- positive progenitors (Fig. 1A, E). The second phase involves the restriction of these progenitors to distinct locations, where superstructures are to be formed (Fig. 1B, F and H). This phase takes place around E12.5-E13.5. During the third phase, which occurs around E13.5-E14.5, the progenitors stop expressing both genes and differentiate into either *Scx*-expressing tenocytes or *Sox9*- and *Col2a1*-expressing chondrocytes (Fig. 1C, G, I and J). Finally, in some scenarios there is also a fourth phase, at which separation of the superstructure from the primary cartilage template occurs and a new auxiliary bone, such as a sesamoid bone, forms (Fig. 1D).

nism for adaptation of the musculoskeletal system to environmental changes and pressures, as modules can be altered or created without the need to rewrite the entire skeletogenic program. This strategy allows for the formation of secondary structures on the bone surface through a designated developmental program (Fig. 1A, B). The incorporation of a joint formation program into the strategy adds another layer of developmental plasticity, as it allows secondary structures to detach from the skeleton and form new auxiliary bones (Fig. 1C).

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The Intervertebral Disc: Working Together in Harmony; Breaking up in Pain

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ABSTRACT

The intervertebral discs are the mobile units of the spine, situated between the vertebral bodies. They provide mechanical motion and dissipate loading during daily activity. While precise assembly of the different units of the spine in development is critical for proper function in postnatal life, molecular and cellular contributions to the formation of the spinal units are not well understood; in particular, the intervertebral disc. With age, the disc begins to degenerate and it is thought that the degenerative process has a significant contribution to symptoms such as back pain and sciatica. The link between intervertebral disc degeneration (IDD) and back pain is well established, but what initiate disc degeneration and progression is not clear. While the general consensus is that it leads to dehydration of the disc, affecting its performance to resist compressive forces and reduced range of motion; how it may begin in development, the genetic and cellular contributions to the formation is not well understood. Our recent findings have provided critical insights into the cellular origin in development and relationship between different pools of cells in the intervertebral disc, and how in the context of the genetic background and environment could lead to pathological outcomes, where the breaking up of this relationship can be painful.

INTRODUCTION

The intervertebral disc (IVD) consists of three integrated components; nucleus pulposus (NP), annulus fibrosus (AF) and cartilage endplate (CEP) (Figure 1). The IVDs connect the vertebral bodies, providing rotation and flexion motions, and dissipate mechanical loads in our daily activities. With aging, there is a gradual failure of IVD function from degenerative processes that often lead to pathological symptoms[1,2].

The NP at the centre of the IVD is a "gelatinous" tissue containing chondrocyte-like cells, producing an extracellular matrix consisted mostly of proteoglycan and type II collagen[3]. This combination of matrix molecules attracts and retains water, providing a highly hydrated environment for disc height maintenance and resisting compressive forces. The AF is a lamellar structure surrounding the NP. The inner AF, adjacent to the NP is a fibrocartilage-like tissue, while the outer AF is more tendon like, with cells expressing many tenocyte (tendon cells) markers such as type I collagen and tenonmodulin[4-7]. The concentric layers of collagen fibers extend and connect to the cartilage endplates (CEPs), a structure that is also part of the vertebral body (VB) (Figure 1). As the IVD is avascular tissue, the CEP is the major route of nutrient and oxygen supply from blood vessels accumulating adjacent to the CEP. IVD integrity and function

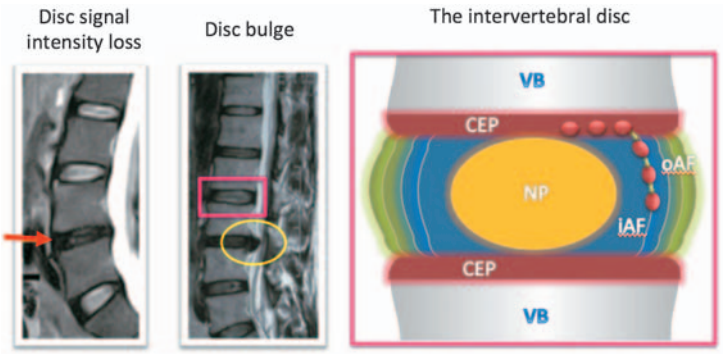

Figure 1. The intervertebral disc. The MRI images on the left represent disc abnormalities commonly seen in patients with symptomatic pain with severe lost of disc signal intensity, indicative of reduced water content (red arrow). Disc bulge represent herniation of the nucleus pulposus (NP) material through the inner annulus fibrosus (iAF) and outer annulus fibrosus (oAF), pinching on the sciatic nerve (yellow circle) leading to sciatica. The right panel is a diagrammatic representation of the region of the red box in the middle MRI image, showing the intervertebral disc situated between two cartilaginous end plates (CEP), the nucleus pulposus (NP) at the center of the disc, surrounded by multiple laminar layers of the annulus fibrosus. The train of cells joining the CEP to the iAF shows the possible contribution of cells within the CEP that can transit to become cells in the iAF for disc maintenance and repair.

rely on the proper cellular function of the disc as a whole. The failure of one is sufficient to induce the breakdown of function as a whole with painful consequences[3]. Understanding the cellular origin, differentiation, and genetic and environmental influences on tissue maintenance are of high clinical relevance in treating symptomatic outcomes.

Cellular origin and differentiation of the nucleus pulposus cells

In embryonic development, while the exact cellular origin of the notochord is unresolved since it originates from cells in the epiblast migrating through the primitive streak, in which the mesendoderm cells are bipotential, the NP is clearly derived from cells of the notochord[8,9]. Using genetic tools in mice to tag and trace notochordal cells with notochord-specific Cre drivers, *Shh*-Cre^{ERT2} [10] and *Noto*-Cre[11], showed that destiny of notochordal cells in the mouse adult spine is indeed the NP. Furthermore, these cells are long lasting in the NP with notochordal descendent cells residing in the adult NP for study periods of up to 9 months[11] and 19 months[10]. However, it is still unclear whether other cells contribute to the NP in postnatal life, as this possibility cannot be excluded from the present studies. Some studies have suggested other cell types are present in aged or degenerated discs with fibroblastic features and the possible presence of macrophages, suggesting other cells can infiltrate into the NP[12-14].

Recently, a detailed analysis of human NP cells showed the presence of distinct pools of cells with specific cell surface markers that could define possible progenitor cells in the NP, and the progressive differentiation towards mature NP cells[15]. Using the tyrosine kinase receptor 2 (Tie2) and disialoganglioside 2 (GD2) as new cell markers, it was shown that Tie2+/GD2-/CD24- cells have characteristics of dormant stem cells; Tie2+/GD2+/CD24- cells have self-renewal potentials, Tie2-/GD2+/CD24- as NP cell progenitors; Tie2-/GD2+/CD24+ as committed NP progenitors; and Tie2-/GD2-/CD24+ as mature cells in the NP[15]. Of clinical relevance is a correlation of progressive loss of these stem/progenitor cell pools with onset/severity of disc degeneration in human[15]. We have further shown that similar correlations exist in mice, in inbreed strains with genetic backgrounds that are more susceptible to intervertebral disc degeneration (unpublished data). Significantly, we propose that maintaining Tie2-/GD2+/CD24+ cells correlates with better outcome that can protect against disc degeneration (unpublished data).

Cellular contribution to the formation and maintenance of the annulus fibrosus

Anatomical and histological analyses suggest the AF is derived from the sclerotome[16-18], supported by a recent genetic study in mice tagging sclerotome cells using the *Tbx18*-Cre[19], that traced cells to the anterior part of the AF and the vertebral bodies, but not in the NP compartment. Involvement of other cell types in development again cannot be excluded.

A mature AF consists of inner (IAF) and outer (OAF) compartments with distinct cell morphology and extracellular matrix composition (Figure 1). How these are formed or partitioned is not clear. In mouse development, the lamellar structures of the AF are not well defined at E13.5-E14.5, but cells begin to orientate concentrically around the NP as formation of the IVD progresses[20]. With growth, the number of lamellae and cells within the AF increase. However, neither the cellular contribution nor the processes of lamellae increment are known.

The presence of progenitor cells identified in IVDs of various animal models[21] suggest these progenitors may contribute to growth, maintenance and repair of the IVD. Of interest, a recent study identified possible "migration routes" of progenitor cells in the outer AF and the involvement of epithelial to mesenchymal transition process (EMT)[22]. As such, this pool of cells is suggested to be a source of progenitor cells for the maintenance of the AF during adult life.

The CEP and AF are tightly connected, analogous to a tendon insertion into bone, forming a "continuous tissue". There is also evidence of "Sharpey's fibers" bridging the two tissues[23,24]. The inner AF inserts directly into the CEP. The junction where the two structures intersect is described as the AF-VB interface[25]. At this interface, there are histological evidence showing column of cells bridging the interface with gradually morphological change from round chondrocyte-like cells to elongated fibroblastic like cells aligned along the laminar structure of the AF[25].

To assess whether these two types of cells are related or share a common origin, we used a Cola10a1-Cre mouse to tag and trace hypertrophic chondrocytes at the cartilaginous junction of the AF-CEP interface, and show that indeed they can become cells of the inner AF (unpublished data). We also showed that this chondrocyte to inner AF transition is enhanced in the experimental model to induce degeneration, suggesting that this replenishment of inner AF cells may play a role in IVD maintenance and repair (unpublished data).

CONCLUSION

While it is clear that there are anatomical and mechanical loading differences between mouse and human; at the cellular level, it is likely to have many similarities in developmental processes, cellular differentiation, and regulatory mechanisms. Furthermore, the enormous repertoire of inbred mice with distinct genetic backgrounds allows the possibility of studying differences in intervertebral disc maintenance and degeneration. The concept of varying healing potentials in different strains of mice can be exploited. In particular, mice with varying healing potential of cartilaginous tissues, which have been reported[26], and some genetic studies performed aiming to identify risk and protective loci[27-30]. Indeed, our analysis of four strains of mice, C57Black and SM/J (poor healers), and MRL and LG/J (good healers) showed clear differences in IVD maintenance and repair potentials (unpublished data). Dissecting the genetic variations between these mice will enable the identification of potential risk and protective factors for disc degeneration, and analyses of the molecular and cellular changes in relation to the progression of NP cell differen-

tiation and AF cell maintenance will provide the relationship between the function and contribution of the different cells in the IVD structure, enabling the disc to work in harmony in the good healer mice, and their failure in poor healer mice leading to destruction and "pain".

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Session V

Epigenetics: Basic and Clinical Implications

Epigenetic Reprogramming in Physiology and Cancer

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Live-cell Imaging of Chromatin and DNA-methylation Dynamics Using Methyl*RO* Mouse

Kinki University
K. Yamagata

Bone-derived miRNA as a Mediator of Cell-cell Communication

Hiroshima University
Y. Takei, T. Minamizaki, H. Yoshioka and Y. Yoshiko

Epigenetic Reprogramming in Physiology and Cancer

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Key words: Epigenetics, gene regulation, non-coding RNA, breast cancer, endocrine therapy, therapy resistance

ABSTRACT

Epigenetic regulation is the mechanism by which gene function is selectively activated or inactivated for cell function. It provides higher-ordered and specified biological information, beyond the genome sequences. To date, DNA methylation, histone modifications and chromatin formation are involved in the molecular basis of epigenome, together with a variety of regulatory proteins and RNAs in the nucleus. These are closely linked to our developmental program in health and diseases, and will produce the emerging scientific and medical discoveries. Our recent study revealed that a cluster of non-coding RNAs are mechanistically involved in endocrine therapy resistance in human breast cancer cells. Furthermore, resveratrol, a kind of polyphenol, was found to repress these RNAs and inhibit the proliferative activity of breast cancer cells which had acquired resistance. These findings highlight the non-coding RNA-mediated epigenetic mechanisms in cancer cell adaptation, which may be diagnostic and therapeutic targets for therapy-resistant breast cancer.

INTRODUCTION

Modifications on DNA and histones are stably maintained for cell identity. In addition, gene activities at each chromosomal locus are controlled by the regulatory elements such as the enhancer-promoter-insulator and further by the higher-order chromatin formation in the nuclear context[1-6]. These epigenetic states are occasionally reset by environmental factors, and may be inherited as the cellular memory.

Breast cancer is one of the most common types of cancer in women[7,8]. In recent years, both early diagnosis and emerging therapies have been improved, but many patients suffer from metastasis and later recurrence of this disease, as a very important issue.

Estrogens and estrogen receptors are the keys to understand the nature of breast cancer cells and the current therapies[9-12]. About 60~70% of breast cancers are estrogen receptor-α (ER)-positive and highly depend on estrogen for cellular growth and survival. Therefore, endocrine therapies, using aromatase inhibitors and anti-estrogen agents, block estrogen action and are clinically effective for ER-positive breast cancers. Unfortunately, these treatments are often followed by disease recurrence because most breast cancers are initially responsive to these therapies but then develop resistances through unknown mechanisms. Since cancer recurrence further causes metastasis and invasion, we need to identify the mechanism of the therapy resistance.

ER-positive breast cancer cells adapt to estrogen deprivation

ER-positive breast cancer cells undergo hormone-independent proliferation after deprivation of estrogen,

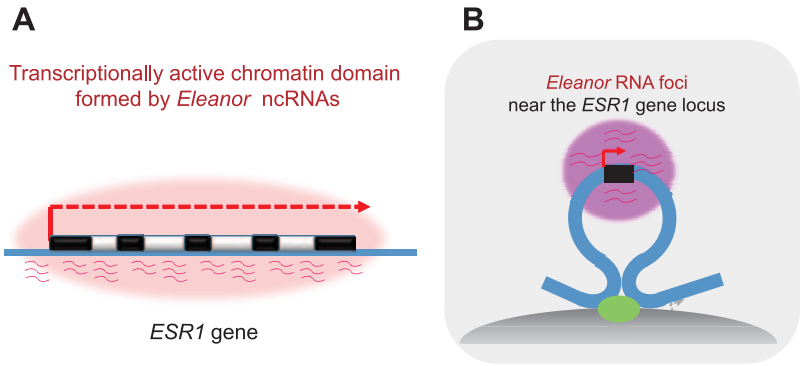

Figure 1. *Eleanor* non-coding RNAs activate the *ESR1* gene locus in ER-positive breast cancer cells under long-term estrogen deprivation.
A. *Eleanor* non-coding RNAs (ncRNAs) are transcribed from the *ESR1* gene locus, and form the RNA foci. B. Based on FISH analysis, *Eleanor* ncRNAs, *ESR1* gene and genomic DNAs are indicated with red, black and blue, respectively (Ref. 13).

leading to endocrine therapy resistance. Up-regulation of the *ESR1* gene is critical for this process, but the underlying mechanisms remained unclear. To investigate processes of the cancer cell adaptation, we used the human breast cancer cell line MCF7 which was ER-positive[13]. These cells can acquire regrowth after long term estrogen deprivation (LTED), similar to cancer cells that become resistant to endocrine therapy. The expression of the ER gene (*ESR1*) was increased during this adaptation in the LTED cells.

At this time, we performed the combinations of transcriptome and fluorescence *in situ* hybridization (FISH) analyses[13]. We found that estrogen deprivation induced a cluster of non-coding RNAs (ncRNAs), which were expressed from a large chromatin domain containing the *ESR1* locus. We named these RNAs as *Eleanors* (*ESR1 locus enhancing and activating non-coding RNAs*). Interestingly, *Eleanor* ncRNAs were produced from the *ESR1* gene locus and were localized at the site of actively transcribed *ESR1* locus, resulting in the formation of distinct RNA foci in the nucleus (Figure 1).

The effects of *Eleanors* inhibition and resveratrol in ER-overexpressing LTED cells

Depletion of *Eleanors* that were transcribed from the putative enhancer of the *ESR1* gene, *upstream (u)-Eleanor*, impaired transcription of intragenic *Eleanors* and *ESR1* mRNA, indicating that *Eleanors cis*-activate the *ESR1* gene[13]. The *u-Eleanor* depletion significantly reduced cell growth. Furthermore, resveratrol, a kind of polyphenol with estrogen-like activity, was found to repress these RNAs and inhibit the proliferative activity of breast cancer cells which had acquired estrogen-independent growth.

These findings uncovered the molecular basis for endocrine therapy-resistant breast cancer, which showed the significant role of a new type of ncRNA-mediated regulation of the *ESR1* gene locus. Thus, *Eleanor* ncRNAs are actively involved in the epigenetic adaptation of ER-positive breast cancer cells via high expression of the *ESR1* gene. Our study emphasizes that the ncRNA-mediated mechanisms in cancer cell adaptation may be diagnostic and therapeutic targets for endocrine therapy-resistant breast cancer.

In addition, *Eleanors*-mediated gene activation represents a new type of locus control mechanism and plays an essential role in the adaptation of breast cancer cells. These findings exemplify one of the novel epigenetic mechanisms involving in cancer cell adaptation to environmental factors through the alteration of chromatin and nuclear context.

CONCLUSION

This study revealed that a cluster of *Eleanor* non-coding RNAs are mechanistically involved in endocrine therapy resistance in human breast cancer cells. Furthermore, resveratrol was found to repress these RNAs and inhibit the proliferative activity of breast cancer cells which had acquired resistance.

ACKNOWLEDGEMENTS & CONFLICT OF INTEREST

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan Science and Technology Agency (CREST). There is no conflict of interest in this study.

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Live-cell Imaging of Chromatin and DNA-methylation Dynamics Using Methyl*RO* Mouse

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Key words: Methyl*RO*, DNA methylation, Epigenetic Dynamics, Heterochromatin, Live-cell Imaging

ABSTRACT

In mammals, DNA is methylated at CpG sites, which play pivotal roles in gene silencing and chromatin organization. Furthermore, DNA methylation undergoes dynamic changes during development, differentiation, and in pathological processes. The conventional methods represent snapshots; therefore, the dynamics of this marker within living organisms remains unclear. To track this dynamics, we made a knockin mouse that expresses a red fluorescent protein (RFP)-fused methyl-CpG-binding domain (MBD) protein from the ROSA26 locus ubiquitously; we named it MethylRO (methylation probe in ROSA26 locus). Using this mouse, we performed RFP-mediated methylated DNA immunoprecipitation sequencing (MeDIP-seq), whole-body section analysis, and live-cell imaging. We discovered that mobility and pattern of heterochromatin as well as DNA methylation signal intensity inside the nuclei can be markers for cellular differentiation status. Thus, the MethylRO mouse represents a powerful bioresource and technique for DNA methylation dynamics studies in developmental biology, stem cell biology, as well as in disease states.

TEXT

In mammals, DNA is covalently modified by methylation of the cytosine base in the CpG dinucleotides and these sites are frequently concentrated in repetitive sequences, such as pericentromeric regions and transposable elements[1,2]. Once these CpG sites are methylated, these are recognized by methyl-CpG-binding domain (MBD) family proteins[3], which then recruit additional protein complexes to these methylated DNA regions to repress gene expression and/or to constitute higher order condensed chromatin structure called heterochromatin to stabilize chromosome structures[3]. Previous studies using DNA methyltransferase knockout mice have revealed that loss of DNA methylation leads to early embryonic lethality (around E9.0), implicating the importance of this epigenetic mark during development[4], and recent studies have indicated that DNA methylation undergoes dynamic changes during mouse embryonic development as well as in tumorigenic processes[5,6]. Although there is a strong need for studying the dynamics of DNA methylation status, there is no appropriate way at present to study this dynamics in living cells or in living organisms. Currently, the major ways to study DNA methylation are bisulfite sequencing[7], methylated DNA immunoprecipitation (MeDIP) combined either with microarray or next-generation sequencing (MeDIP-seq)[8], and immunohistochemistry[9,10]. However, all techniques require fixation of the cells and hence make it almost impossible to study the dynamics of this pivotal epigenetic mark. Previously, Yamagata and colleagues have succeeded to visualize DNA methylation status by injecting green fluorescent protein fused MBD and nuclear localizing signal of MBD1 protein (EGFP-MBD-NLS) mRNA to living mice zygote. By use of this method, they have succeeded to visualize the DNA methylation status in preimplantation mouse embryos and found that DNA hypomethylation as a key signature which distinguishes germ cells from somatic cells[1,11]. Importantly, this method not only can trace epigenetic changes in real time but also in single-cell resolution which was impossible with conventional methods. To apply this methodology to other cellular differentiation processes, we have knocked-in *RFP (red fluorescent protein) fused MBD* and NLS reporter probe (mCherry-MBD-NLS) gene into *ROSA26* locus and generated a mouse strain which captures global DNA methylation status in living condition.

Generation of Methyl*RO* mouse

We chose *ROSA26* locus because this locus is well known for its ubiquitous and uniform expression in mice[12,13] and widely used in reporter gene expressions[14,15]. We decided to fuse RFP because they have better signal to noise ratio compared to GFP. By using conventional gene targeting method, *mCherry-MBD-NLS cDNA* was successfully knocked into *ROSA26* locus with high efficiency (28 out of 96 clones). In excellent agreement with previous studies[1,11,16], mCherry-MBD-NLS probe localized to pericentromeric regions and formed foci in interphase nucleus in ES cells (Figure 1A). Importantly, mCherry-MBD-NLS probe expressed from *ROSA26* locus was uniform compared to probe expressed from other promoters, such as CMV and CAG. We then

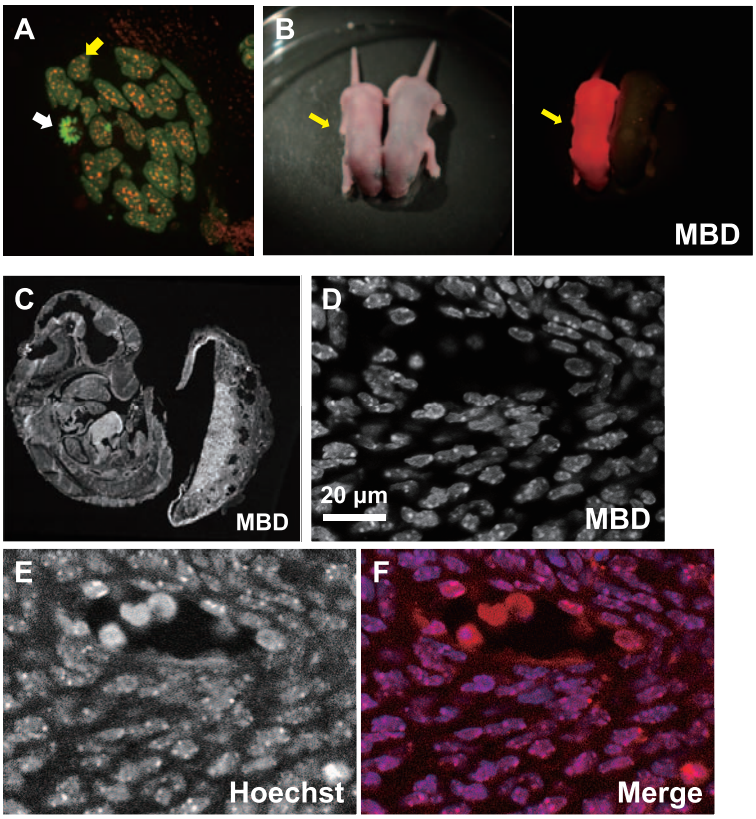

Figure 1. Generation of Methyl*RO* mouse.
(A) Fluorescent image of ES cells introduced mCherry-MBD-NLS probe gene in *ROSA26* locus. The probe (red) localized to pericentric regions of metaphase chromosomes (white arrow) and formed foci in interphase nuclei (yellow arrow) in ES cells. Green signals represent nuclei and chromosomes labeled by histone H2B-EGFP. (B) Mouse expressing mCherry-MBD-NLS probe in the whole body is viable and healthy. We named this mouse Methyl*RO* (methylation probe in *ROSA26*). Left and right panels are blight and fluorescent images, respectively. (C-F) Section of an E12.5 MethylRO mouse fetus. Somatic cells near the tail were taken with a x40 objective lens. The mCherry-MBD-NLS probe (MBD) within somatic cell nuclei exhibited a typical heterochromatic foci pattern that was also marked by Hoechst dye (Hoechst).

generated chimeric mice from these ES lines, and males were crossed with C57BL/6 female mice to obtain *ROSA26-mCherry-MBD* heterozygous reporter mice. As shown in Figure1B the mCherry-MBD-NLS expressing mice, which we named 'Methyl*RO*/métʃirou/ (Methylation probe in *ROSA26* locus)', were viable and fertile, indicating that the MBD probe expressed from *ROSA26* locus has no obvious toxicity to mouse development, survival and fertility.

Expression profiles of *mCherry-MBD-NLS* probe in embryonic and adult mice organs and tissues

We next examined various tissues and organs of Methyl*RO* mouse. As expected, RFP signals can be identified in all tissues examined (data not shown), confirming that this probe is ubiquitously expressed within both fetal and adult mouse bodies. To gain further insights on how this probe is functioning inside the nuclei, we made tissue sections of E12.5 embryo (Figure C) and observed in closer details. Importantly, we observed a typical pattern of MBD probe inside the nuclei (Figure 1D-F), implicating that this probe is functional inside the mouse body. Furthermore, MBD probe co-localized with dotted Hoechst stained nuclei, which marks heterochromatic regions, and this clearly indicates that this probe is recognizing heterochromatin within mouse body.

Live-cell imaging of Methyl*RO* embryos

Finally, we performed live-cell imaging of preimplantation embryos collected from Methyl*RO* mice. As shown in Figure 2A, we have successfully imaged global DNA methylation dynamics starting from 2-cell stage until late blastocyst stage embryos. This was not possible when we injected EGFP-MBD-NLS probe mRNA into zygotes, which is our previous method, because the signal starts to decrease from 8-cell stage onwards, and extremely difficult or impossible to detect signals in blastocyst-stage embryos.

We then carried out live-cell imaging analysis of ES cell derivation process using MethylRO embryos. According to our previous methodology[17], morula stage embryo collected from MethylRO mouse was placed on

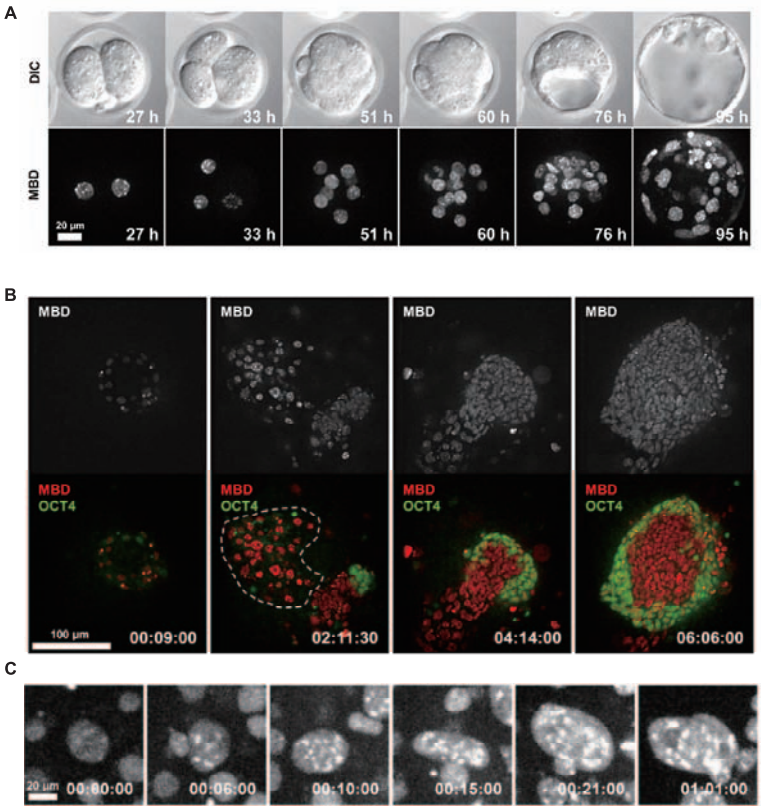

Figure 2. Global DNA methylation patterns change dynamically during preimplantation mouse embryonic development and cellular differentiation process during ES cell derivation.
(A) Time-lapse imaging of Methyl*RO* preimplantation embryo was performed using 30x silicone oil immersion objective lens equipped confocal microscope. Images were taken every one hour from zygote (0 h) to blastocyst stage (119 h). mCherry-MBD-NLS protein becomes visible from 2-cell stage embryos (19 h). (B) Time-lapse imaging of ES-derivation process using MethylRO (red) and Oct3/4-EGFP (green) -double-reporter embryos. Oct3/4-EGFP marked pluripotent epiblast cell lineages that eventually become ES cells. Nuclei surrounded by the dotted line indicate trophoblast giant cells. (C) Dynamic nuclear remodeling of trophoblast giant cells. Note that heterochromatin foci became apparent labeled by mCherry-MBD-NLS probe during the differentiation process. The time after starting the observation is indicated at the bottom right (day: hour: minute).

top of feeder cells, cultured in conventional ES cell derivation medium, and imaged every 30 minutes up to 7 days. To label the pluripotent epiblast cell lineage, we have generated Oct3/4-EGFP knock-in mouse using the previously established targeting vector construct[18] (Figure 2B). Intriguingly, although heterochromatic foci were vague in the beginning, these became distinct during the course of derivation. These data clearly indicated that epiblast cells indeed have undergone epigenetic and heterochromatin remodeling during ES cell derivation process. In parallel to ES cell derivation process we also captured dynamic nuclear remodeling of trophoblast giant cells during its emergence (Figure 2C). Strikingly, heterochromatic foci of trophectodermal cells became stronger in time-dependent manner along with nuclear size increase. These observations suggest the possibility that not only the DNA methylation status itself but also heterochromatin dynamics and stability could be a marker for the cellular differentiation status. To our knowledge, this is the first live-cell imaging of epigenetic dynamics during differentiation. Thus, we have demonstrated that MethylRO can capture DNA methylation dynamics during differentiation in living cells and in mice. Therefore, Methyl*RO* mouse will be a powerful resource to study the chromatin and epigenetic dynamics in in developmental biology, stem cell biology, as well as in disease states.

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Bone-derived miRNA as a Mediator of Cell-cell Communication

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Key words: Bone metabolism, matrix vesicle, microRNA and cell-cell communication

ABSTRACT

The field of epigenetics has recently been at the forefront of multiple studies in both the basic science and clinical applications. Non-genetic alternations including DNA methylation, histone modifications and non-coding RNA associated gene silencing initiate and sustain epigenetic changes. A microRNA (miRNA), our focus in this study, is a small non-coding RNA and functions as a RNA silencer and post-transcriptional regulator of gene expression. Intensive studies indicate that miRNAs are secreted and delivered to recipient cells through the bloodstream or microenvironment, and thereby act as mediators of cell-cell communication. Here, we first identified a large number of miRNAs secreted by osteoblasts and clarified that one of miRNAs is involved in bone metabolism. Our findings prompt us to develop a new therapeutic approach for the treatment of skeletal disorders.

STATEMENTS

miRNAs are genomically encoded small non-coding RNAs, and the genes encoding miRNAs are much longer than their end products (mature miRNAs). Through miRNA processing machinery, small miRNA molecules comprised of 20-25 nucleotides are produced as mature miRNAs. miRNAs partially target complementary one or more mRNAs to downregulate gene expression in different manners, such as translational repression, mRNA cleavage, and deadenylation[1]. To date, more than 1,800 miRNAs have been identified in humans (miRBase ver. 21, http://www.mirbase.org/).

Bone, a type of supporting tissue, enables mobility, stores minerals, and includes marrow cells. This organ is metabolically active and maintained by a precise balance between bone resorption and formation. Osteoclasts are the multinucleated giant cells derived from a monocyte-macrophage lineage and equipped with phagocytic-like mechanism to resorb bone. Osteoblasts are the cells of mesenchymal origin and responsible for bone formation. Osteocytes are terminally differentiated osteoblasts embedded in bone and maintain the bone architecture. The vascularized and innervated bone marrow includes mesenchymal and haematopoietic lineages. Osteoblasts produced osteocalcin which is undercarboxylated and controls insulin sensitivity through the endocrine system[2]. Also, osteoblastic cells comprise haematopoietic and cancer stem cell niche[3-5]. Thus, bone contributes to many aspects of skeletal and extraskeletal development, homeostasis, aging and so on.

The roles of miRNAs in bone metabolism have been explored. Conditional deletion of the pre-miRNA cleavage enzyme Dicer in osteoblast lineage cells alters osteogenic development[6], suggesting the involvement of miRNAs in bone formation. miR-204 and miR-31 are targeted to *Runx2*[7] and *Sp7*[8], respectively. These gene products are essential transcription factors for osteoblastogenesis. miR-124 inhibits osteoclast differentiation by downregulating *Nfatc1*, a master regulator of osteoclast differentiation[9]. Growing evidence indicates that RNA-induced silencing complexes and/or exosomes including miRNAs are secreted and transferred to recipient cells and that secreted miRNAs act as mediators of cell-cell communication[10,11]. Based on these backgrounds, we

Table 1. Representative miRNAs secreted by mouse osteoblastic MC3T3-E1 cells.

miRNAs	miRBase Accession	Active sequences	Relative levels	Representative targets
mmu-miR-3960	MIMAT0019336	CCCCCGCCTCCG	8.104	*Bcl11a, Hoxa2*
mmu-let-7b-5p	MIMAT0000522	AACCACACAACCTACTACC	5.576	*c-Myc, Dmp1, Muc1*
mmu-miR-494-3p	MIMAT0003182	GAGGTTTCCCGTGTA	5.542	*Fgfr2, Rock1*
mmu-miR-125b-5p	MIMAT0000136	TCACAAGTTAGGGTCTC	5.432	*Akt1, Prdm1, Irf4*
mmu-let-7c-5p	MIMAT0000523	AACCATACAACCTACTACC	5.161	*Bmp5, CDC25A*
mmu-miR-199a-3p	MIMAT0000230	TAACCAATGTGCAGACTACT	4.441	*Hif-1α, mTor, Vegf-a*
mmu-let-7i-5p	MIMAT0000122	AACAGCACAAACTACTACCTC	4.425	*Bmp4, Tlr4*
mmu-miR-21a-5p	MIMAT0000530	TCAACATCAGTCTGATAAGC	4.121	*BmprII, Mkk3, Sox2*
mmu-let-7a-5p	MIMAT0000521	AACTATACAACCTACTACCT	4.101	*Chd4, E2f2, Stat3*
mmu-miR-22-3p	MIMAT0000531	ACAGTTCTTCAACTGGCAG	3.864	*Bmp7, Ccna2, Tcf7*

provide evidence for a new form of intracellular communication in bone.

We performed miRNA microarray analysis on extracellular components from mouse osteoblastic MC3T3-E1 cells. Of miRNAs identified, over 50 miRNAs were identical to human counterparts. We chose 10 miRNAs with abundant amounts and validated target genes (Table 1). As expected, one of miRNAs was selectively transferred to recipient cells and downregulated target gene, resulting in defective cell activities. We will provide experimental data on the roles of this miRNA in bone both *in vivo* and *in vitro* and discuss in detail.

REMARKS

Bone act as an endocrine organ to orchestrate human body. Thus, the appropriate interventions to cell-cell communication in bone may be necessary to integrate therapies for both the skeletal and extraskeletal consequences. We expect that this approach is greatly facilitated by our findings in bone-derived secreted miRNAs.

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Session VI

Challenges and Perspectives in Dental Education

Improvement and Substantiality in Dental Education

Higher Education Bureau
S. Terakado

Achievement of a Sophisticated Dental Education Programme in Collaboration with Niigata University, Tohoku University, and Hiroshima University

Tohoku University, Niigata University, Hiroshima University
T. Tenkumo, Y. Takeuchi, G. Hong, Y. Ishida, R. Takeishi, S. Mimura, T. Shuto, H. Oka, K. Yoshiba, N. Fujii, K. Tsuga, T. Taji, N. Takahashi, K. Ono, K. Uoshima, H. Nikawa, K. Sasaki, M. Sugai and T. Maeda

The Education of Dental Technology in Taiwan

Taipei Medical University
C.K. Lin, W.F. Lee and C.T. Lin

Quality Assurance in Dental Education, "Dentist & Shikaishi"

Tokyo Medical and Dental University
J. Tsuruta

Improvement and Enhancement of BioDental Education and Research in Hiroshima

Hiroshima University
K. Kato and M. Sugai

Improvement and Substantiality in Dental Education

S. Terakado

Director, Medical Education Division, Higher Education Bureau

Hiroshima University Faculty of Dentistry 50th Anniversary

Improvement and Substantiality in Dental Education

October 24, 2015

Shigechika Terakado
Director, Medical Education Division, Higher Education Bureau

*Translated English is not officially endorsed.

Inquiry Research Council for Improvement and Substantiality in Dental Education

[Purpose]
To investigate and study the specialized matters concerning improvement and substantiality in dental education, and compile reports if necessary.

[Reports Thus Far]
- First phase report (January, 2009)
- Follow-up summary based on the first phase report (May, 2011)
- Follow-up summary based on the first phase report (December, 2012)
- Proposal and request (February, 2014)

1

First Phase Report, Outline and Overview 1

1 . Assure Clinical Ability

- Insufficient goal setting and evaluation/assessment
- Insufficient clinical training/clinical clerkship hours (caused by difficulty in maintaining patient cooperation with student care and the national board preparation)

- Clarify outcomes at their graduation, and specify requirements and the unit number in clinical clerkship.
- Implement post clinical clerkship OSCE.
- Promote clinical training utilizing external institutions

2

First Phase Report, Outline and Overview 2

2 . Implement Systemic Dental Education

- Non-exclusivity of university features
- Disparity of clinical trainings and classroom learning after CAT (common achievement test)

- Organize systemic dental education course thoroughly and conduct strict grade evaluation and grade promotion judgement.
- Revise model core curriculum for dental education.
- Introduce third-party evaluation (system) to assure dental education quality.

3

First Phase Report, Outline and Overview 3

3 . Secure Admission Excellency with regard to Social Demands

- Decline in function of entrance examinations
- Decreasing attractiveness of dentistry as an occupation, caused by the excessive number of dentists

- Clarify admission policies, open information for entrance examination to public.
- Device a plan to distinguish students' aptitudes by enhanced interviews, liaison with high schools, etc.
- Reconsider enrollment number for universities that have difficulties in maintaining admission excellency or having low pass rate in the national board.

4

First Phase Report, Outline and Overview 4

4 . Cultivate Researchers, Pioneering Future Dental Medicine

- Absence of research integrated in basic science and clinical dentistry
- Necessitate research mind-set from undergraduate level

- Expand opportunities for undergraduate students to engage in research Activities.
- Clarify purpose and content in graduate school education (in dentistry) to correspond with the outcomes (training for clinicians or researchers).
- Construct a base to cultivate/train global young researchers regardless of the university unit.

5

Follow-up summary (Abstract) May, 2011

[Current Situation]

All dental schools in Japan undertake/start plans for improvement based on the first phase report, yet some are required to reflect the ongoing situations and change or improve their current educational courses and contents, admission excellency, and enrollment numbers.

[Room for Improvement]

①Improvement and substantiality in clinical clerkship (cc), outcome setting and evaluation for clinical ability
②How to support those who repeat a school year
③Guarantee for admission excellency
④Periodic examinations
⑤Training/cultivation for researchers

6

Follow-up summary (Abstract) December, 2012

[Current Situation]

Results derived from a follow-up in 2010 became apparent, yet some are still required to reflect the ongoing situations and change or improve their current educational courses and contents, admission excellency, and enrollment numbers.

[Room for Improvement]

①Excessive numbers in enrollments
②Criteria for treating patients by oneself
③Improvement and substantiality in cc, outcome setting and evaluation for clinical ability
④Guarantee for admission excellency
⑤Improvement in students' academic ability and pass rate for the national board, and decrease in the ratio of students who repeat a school year
⑥Training/cultivation for researchers
⑦Disclosure of information concerning educational activities
⑧Features of dental education at each university

7

Proposal and Request (Abstract) (2014.4) 1

1. **Substantiality in clinical clerkship**
2. **Dental education with regard to various dental needs**
3. **Disclosure of information concerning educational activities**
4. **Introduction of the accreditation system in dental education**
5. **Enrollment number control**

8

Proposal and Request (Abstract) (2014.4) 2

1. Substantiality in clinical clerkship

○ Please make sure to maintain substantiality in clinical clerkship to assure clinical ability.

2. Dental education with regard to various dental needs

○ In order to gain trust and understanding in dental education from public, and to increase the field for dentists to prosper, it is necessary to establish faculties of dentistry that serve as advocates of social reform.
○ Please make efforts in cultivating dentists who can devote themselves to various needs dentistry, and in solving global problems applying each university's qualities.

9

Proposal and Request (Abstract) (2014.4) 3

3. Disclosure of information concerning educational activities

○ Information concerning educational activities is important for applicants and undergraduate students. Please disclose this information, for example on your HP, and engage in appropriate action in accordance with the social evaluation.

Tuition fee based on the admission requirement, passing rate of the national board, clinical clerkship, outcomes, quality assurance, etc

10

Proposal and Request (Abstract) (2014.4) 4

4. Introduction of accreditation system in dental education

○ To aim for further improvement and guarantee that Japanese dental education is up to par the international standards, introducing the accreditation system in dental education is necessary.
○ `Research on the Establishment of Dental Accreditation System in Japan` has started since 2012 subsidized by mext fund.
This council expects its outcome and supports this project.
○ (To faculty of dentistry) Please understand and cooperate with this project and the introduction of the accreditation system in dental education.

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5. Enrollment number control

○ As excessive or decreased enrollment number would cause decline in dental education, please maintain student quotas. Please secure admission excellency by setting appropriate enrollment number and improving admission selection by referring to the cabinet decision on dentist number control.

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Future Efforts/Approaches by MEXT

1. Follow-up based on the first phase report

Investigations from November 2014, site visit from June to September in 2015, and compile reports by March 2016.

2. Support with a grant-in-aid

- **`Research on the Establishment of Dental Accreditation System in Japan` project from 2012-2016**
- **'Problem-solving Oriented Training Program for Advanced Medical Personnel' project (Representatives : Okayama university, Hokkaido university etc.) from 2014-2018**

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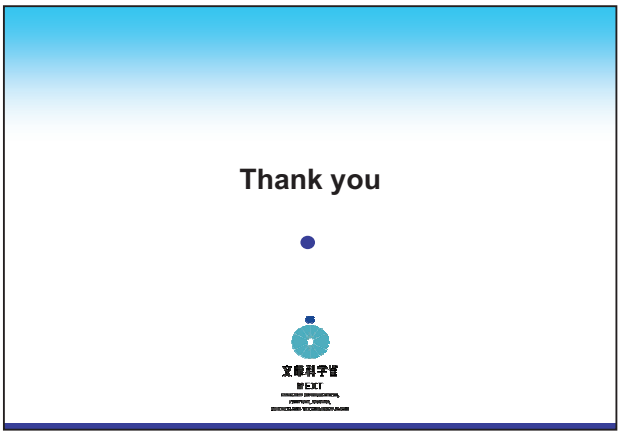
Thank you

Achievement of a Sophisticated Dental Education Programme in Collaboration with Niigata University, Tohoku University, and Hiroshima University

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Key words: inter-university collaboration, undergraduate clinical education, clinical competency, cultivation of competent dentist

INTRODUCTION

Currently, the improvement of the quality of the undergraduate education system is a main issue in Japanese dental education. Especially, the decline of undergraduate students' dental clinical competency due to the shortage of educational resources for current dental education and the resultant inefficiency of ensuring excellent students or leaders for the next generation, as well as the disparity between dental universities in terms of their performance on accredited assessments, are pointed out as urgent issues. Niigata University, Tohoku University, and Hiroshima University have collaborated to work on these issues as the basis of the programme 'Sophistication of Dental Education Programmes Utilising Inter-School Relationships', supported by a MEXT grant since 2012. In this programme, we aim to assure the quality of dental clinical competency and to adapt the Japanese dental education system to global standards thorough the following programme missions: **1) to promote dental clinical competency for current needs, 2) to foster promising dental researchers, 3) to cultivate internationality and sociality, and 4) to assess the educational system for undergraduate dental students.** To achieve these missions, we developed original, individual education sources for the purposes, shared them amongst the three universities, and attempted the standardisation of dental education. In the following chapters, we will describe the achievements of these missions and our further prospect.

1. FOSTERING DENTISTS WITH CLINICAL COMPETENCY FOR CURRENT NEEDS

The increase of the average life expectancy has induced a rise in the number of elderly patients visiting dental hospitals with complicated oral and systemic disorders. This change has promptly demanded a high quality of dental treatment, which has made it difficult to ensure there are patients available who are suitable for taking part in the clinical education of undergraduate dental students in Japan and resulted in a shortage of (volunteer) patients for clinical education. Therefore, more effective clinical education programmes are needed to compensate for the dental clinical competency of undergraduate students and to deliver a high quality of dental services to the public.

· Clinical simulation training utilising an integrated dental training model (Tohoku University)

Tohoku University has developed clinical simulation training by using a dental training jaw model, based on an original model provided from Niigata University. The model can be used for comprehensive clinical treatments, including endodontic, periodontic, and prosthodontic procedures using a mannequin. We prepared scenarios such as the patient's information and history, X-ray images, periodontal chart, etc., for the clinical statement of the model. An original scoring sheet based on a rubric assessment (rubric sheet) and a questionnaire concerning this training course were used to evaluate students' products and this course, respectively. The training course was conducted by six departments in the school and offered for preclinical undergraduate students. The rubric sheet and questionnaire were collected to assess the validity. This training course could promote the effective compilation and organisation of previously learned knowledge and skills, and it is considered to be appropriate for the guidance of basic clinical training. On the other hand, there was a wide gap in scores among the instructors in some t.asks, suggesting that further

modification of the rubric score sheet is needed, and the instructors should adopt more uniform views.

· Development of software for dental diagnosis simulation (Tohoku University)

We developed the original diagnosis simulation software programme, which incorporates a practical method of thinking, to improve the students' diagnostic competence. The participants selected some exploration items that they thought were necessary for a clinical site, then diagnosed patients using the collected information and drew up treatment plans. The participants could learn the comprehensive process of clinical necessary explorations and dental diagnosis by selecting the explorations by themselves and establishing the diagnosis and treatment plan on the software. We evaluated the validity of the software through a questionnaire given to the participants. The questionnaire results showed that the programme was effective for improving the undergraduate students' competency in diagnosis and treatment plans. We are now increasing the number of cases and trying to find an appropriate degree of difficulty for students' effective learning.

· Development of an objective-structured evaluation method for clinical competency utilising a standardised dental model (Niigata University, Tohoku University, and Hiroshima University)

The evaluation system for the dental clinical technique of undergraduate students is considered necessary for the national examination for a dental license. However, there is no standardised evaluation system yet. Therefore, we developed an objective-structured evaluation method using a standardised dental model and the special evaluation method, beginning in 2013. The model has teeth with dental calculus and dental caries, which require composite resin restoration, endodontic treatment, or extraction. A special evaluation sheet for the successful treatment of dental morbidity was prepared. Trial tests were carried out twice. In each test, approximately 30 students and residents (10 fifth-year students, 10 sixth-year students, and 10 residents) at each university completed the specified dental tasks like composite resin restoration, endodontic treatment, or tooth extraction, etc., within a limited time (total of three hours). A prepared evaluation sheet was used for self-evaluation and evaluation by instructors who had more than eight years of experience as dentists. The evaluation was performed within five minutes per subject. Meanwhile, the validity of the developed evaluation system was assessed through statistical analysis and a questionnaire. The results of the statistical analysis showed that the residents received higher scores on the evaluation compared to the students. The questionnaire results showed that the developed dental model was well accepted by more than 90% of students and residents. These results suggest that the evaluation system has adequate validity and can be useful in the national examination for a dental license. We are planning to introduce a digital assessment to increase the objectivity of the evaluation as the next step.

2. CULTIVATION OF PROMISING DENTAL RESEARCHERS

· Lectures on the latest dentistry (Niigata University, Tohoku University, and Hiroshima University)

Each university has original and special departments such as swallowing rehabilitation at Niigata University, disaster dental medicine and dentistry-engineering collaboration at Tohoku University, and dental globalisation education and new dental technical education at Hiroshima University. While these departments provide valuable lectures within the university, the lectures are not systematically available at other universities. In this project, collaboration lectures were carried out for dental students to acquire the latest dental knowledge. A total of 26 collaboration lectures (6 lectures in 2014 and 10 lectures in 2015) were provided for fifth-year students, and they were video-recorded for (video) on demand release in the future. The evaluation was carried out via a questionnaire filled out by the students, 86.9% of whom gave positive answers about the lectures. Our goal in this project is to set up these collaborative dental education courses to be regular courses for culturing competent dentists.

· BioDental programme at Hiroshima University (Hiroshima University)

Hiroshima University has had a BioDental education programme based on biology since 2008 to cultivate biodentists, oral health managers, and oral engineers for the development of promising dental professionals and researchers. The BioDental programme was held for students at Niigata University and Tohoku University who were past their fourth year of studies, to standardise the education programme at the three universities. In the BioDental education, students conducted the basic practice for cell culture and clinical practice for the evaluation of oral function using ME machines, and techniques for digital dentistry using the CAD/CAM system and 3D printer, over a period of four days. The evaluation was carried out through a questionnaire and comparative analysis of self-evaluations between the pre- and post-mission. This evaluation by external students strongly facilitated the standardisation of the BioDental programme.

3. CULTIVATION OF INTERNATIONALITY AND SOCIALITY

In the rapidly globalising era, the acquisition of internationality and sociality is prerequisite for clinicians and researchers to be active in the world. Fostering dentists who hold an international view is an important issue in dental education for the next generation.

A short stay programme was carried out, in which approximately 15 students (in their first to sixth year of study) visited the Faculty of Dentistry, Khon Kaen University, Thailand, and stayed there for 10 days. The students were divided in three groups and observed the dental clinic in accordance with the prepared schedule. They communicated directly with instructors and students of the host university in English and experienced the dental education and cultural differences. The students discussed points that were similar and different from Japan as well as the reasons. The achievements of this short stay were presented at the student session of

JFDA (2015).

4. ASSESSMENT OF THE EDUCATIONAL COMPETENCIES OF UNDERGRADUATE DENTAL STUDENTS (Niigata University, Tohoku University, and Hiroshima University)

· The organisation of the education programme and quality assurance system by instructors at each university using faculty development (FD)

To provide high quality education to undergraduate students, instructors have to resolve differences in viewpoints among instructors and collaborate on current issues in dental education. We have held faculty development (FD) once a year to improve quality assurance at a personal level and to introduce the education technique based on advanced education theory. These FDs have dealt with common themes at the three universities as follows: 'The evaluation of learning achievements' in 2014 and 'Issues and directions in the accreditation evaluation of dental education in Japan' in 2015.

· The organisation of the systematic education programme and quality assurance system: Platform committee and external evaluation committee

We have periodically held platform meetings and received external reviews by a commission composed of stakeholder commissioners (JEDA) and external experts for the assurance of the quality of this project. In addition, we held the external evaluation committee (Tokyo) (http://www.dent.niigata-u.ac.jp/renkei/report/2014/1003/) and the symposium of the interim report for standardisation in Japanese dental education (Niigata) (http://www.dent.niigata-.ac.jp/renkei/report/2014/1003/). The reviews for this project were edited and published in a book. Furthermore, platform committee meetings were held to promote collaborative education at the three universities three or four times per year, and the management and results of each mission were evaluated and discussed. This self-evaluation in the platform committee meeting and reviews from external experts or stakeholders efficiently facilitated the PDCA cycle function and promoted our education programme and quality assurance system.

· Development of the standardisation of dental education with information transmission

The achievements of these projects have been published in international and national conferences such as JEDA and ADEE. The leaflet was published in English and distributed to ADEE, King's College University, UK, and dental schools in Southeast Asia. Furthermore, we set up the homepage of Niigata University (http://www.dent.niigata-u.ac.jp/renkei/), where the contents of this educational programme and its achievements are available.

The Education of Dental Technology in Taiwan

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ABSTRACT

The "The Dental Technicians Law" was passed on 23th January, 2009 certifying the national licensing of dental technologists. In order to implement the dental technicians act, regulations for senior professional technician examinations, and the regulations for special of for dental technicians was released on 29th March, 2010. The dental technician examination is divided into 2 parts: a written test and an on-site test. There are two subjects for the on-site test: dental morphology and carving, and full mouth removable denture arrangement. A new era for the education of dental technology in Taiwan has begun.

There are four schools of dental technology located in Taiwan, one in the north, one in central Taiwan, and two in the south. In southern Taiwan are found the Department of Dental Laboratory Technology at Min-Hwei College of Health Care Management (program started from September 2007) and Shu-Zen Junior College of Medicine and Management (program started from September 2006). These two schools have five-year programs consisting of three years of high school and two years of professional dental laboratory technology education. These schools focus on traditional dental technology skills.

The Department of Dental Technology and Materials Science at Central Taiwan University of Science and Technology is the oldest dental technology school in Taiwan. The program here started in 1981 and was initially a five-year program similar to the two schools mentioned above. Currently it has a four year undergraduate program and a master's course. Most dental technicians in Taiwan graduated from this school.

While these three dental technology schools are supervised by the technological and vocational education system under the Ministry of Education, the School of Dental Technology at Taipei Medical University, founded in 2007, is the only dental technology school within a medical university and is supervised by the higher education system under the Ministry of Education. The College of Oral Medicine at Taipei Medical University (COM-TMU) is one of the largest dental colleges in Taiwan. COM-TMU offers innovative educational, clinical, research, community service, and industrial connections, and is recognized by various field professionals.

We have resources from three adjunct university hospitals: TMU hospital, Wan Fang hospital, and Shuangho hospital. Currently we have a four year undergraduate program and are applying for a master's course. COM-TMU was established to determine the necessary facilities, equipment, and all-inclusive digital patient records to facilitate comprehensive treatment planning and efficient delivery of oral health care at the highest level of quality using digital dental technologies.

The School of Dental Technology at Taipei Medical University (SDT-TMU), aims for a combination of academics, art, science, and technology. SDT-TMU instructs students in professional knowledge of dental technology and guides them to graduate school. Concerning professional skills and certificates, SDT-TMU educates students starting from basic dental technology and finishes with new digital oral engineering. This includes biomechanics, dental materials, oral function and esthetics, and an understanding of design and manufacture using rapidly expanding digital processes and new dental materials. We encourage international and domestic dental practice. In addition, SDT-TMU expects students to perform research and pursue higher degrees. There are three course tracks that have been developed to enable students to know what they can learn from SDT-TMU programs: traditional dental skills training, preparation for graduate school, and improving digital oral engineering ability.

Many students from SDT-TMU take the student research course and propose their projects to the Ministry of Science and Technology. During this course, the students learn how to find research articles, design experiments, and perform research, allowing them to pursue higher degrees. Currently, six students are running their student research projects and will present their findings early next year.

In order to continue improving the education quality of SDT-TMU, comments from alumni, dental technicians, students, faculties, and friends from sister schools are discussed. The accreditation processes for SDT-TMU is in progress. Excellent performances from alumni, students, and faculty are the key to ensure the SDT-TMU maintains its status as one of the best dental technology schools in the world.

Quality Assurance in Dental Education, "Dentist & Shikaishi"

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ABSTRACT

"Quality Assurance" is a communication tool between the public and our dental education. In the beginning of 21st century, dental educators and other stakeholders in dentistry took active efforts to establish a scheme to assure quality of dental education in Japan. The environment surrounding dental education has been changed as a result of globalization. The accreditation scheme is a key for the mutual recognition of dental license. In the EU, free movement of dentists are guaranteed by the Directives. The Association of Dental Education in Europe has a role to harmonize and converge dental education to create desirable environment for students, patients and the public. As a result of the ADEE activities, the "Profile and Competences for the graduating European dentist—update 2009" has been issued for over 200 dental schools in Europe. In ASEAN, the Ministers signed the Mutual Recognition Arrangement on Dental Practitioner to facilitate the mobility of dental service professions in 2009. Following this arrangement, The South East Asian Association for Dental Education started to discuss their common core competencies for ASEAN dentist. Sharing common competencies and profiles of the dentist can be a basic element for mutual recognition. In medical education, ECFMG in U.S. announced their policy change in 2010, it is that only international medical graduates from accredited medical schools can apply for ECFMG certification. This requirement will be in effective in 2023, however, those medical schools, which has not had the appropriate accreditation started to take a plan to change their curriculum to meet the standard. The accreditation scheme and the standard have been an internal tool for quality assurance in a country until today. If the appropriate accreditation scheme and the standard are implemented to Japanese dental education, a graduate dentist "Shikaishi" may become "Dentist" in other countries in the future.

INTORODUTION

In our society, "Quality Assurance" is getting popular and it influences our life very much. For the last dozen years or so, dental educators and other stakeholders in dentistry took active efforts and contributed to establish a scheme to assure quality of dental education in Japan. For that purpose, the Japanese Model Core Curriculum for Dental Education (JMCCDE), the Common Achievement Test (CAT) which is based on contents of JMCCDE are successfully implemented to dental undergraduate curriculum and they brought meaningful outcomes for the public[1]. It became possible for dental schools to design their dental curriculum with JMCCDE as the standard for Japanese dentist "Shikaishi" and to evaluate dental students' competencies for patient treatment by CAT, CBT & OSCE. Nowadays, a scheme of JMCCDE and CAT becomes fundamental elements like the "Air" for us to spend our life in a field of dental education. A main objective of implementing JMCCDE was to promote more chances for students to have their own patients during a clinical period. It meant that the objective was to strengthen students' clinical competencies at graduation time and to foster a qualified Japanese dentist "Shikaishi" who can work for public and patients safely and independently. For a decade, a lot of activities have been done to introduce new schemes for dental education. Recently, a new accreditation scheme is discussed to improve further quality assurance.

Quality Assurance for dental programs in Japan

In a field of dental education in Japan, especially in undergraduate dental education, an educational institution is one of the most important targets for quality assurance. Dental schools in Japan provide dental programs to foster future dentists "Shikaishi" licensed by the Ministry of Health and Labour, Welfare (MHLW). The outcome of 6-year program is regarded as the competencies and the profile of a new graduate dentist, and dental schools are required to show the evidence how much students learn and perform their skill and behavior in their scheduled programs. In 2001, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) published the Japanese Model Core Curriculum for medical and dental education and it suggested a way of reforming conventional dental curriculum to foster talented students with clinical competencies. JMCCDE will be a kind of the standard for dental education in Japan. A dental school designs a program for 6 years by following items in JMCCDE. And all dental students have to pass the Common Achievement Test, CBT & OSCE, to start treatment of their patients in the clinical period. It is placed at the end of 4th year or the middle of 5th year in the dental programs. CAT can be one of the quality assurance schemes by evaluating students' clinical readiness. In this point, CAT has a role of a summative examination similar to the National Examinations for Dental Practitioner, which is held in the end of 6th year by the MHLW. In recent years, a pass rate of the National Examinations for Dental Practitioner, is around 70%, it seems to be low rate as qualification examination though

the pass rate varies among 29 dental schools and some dental schools seem to have some problems of graduate's competencies. To control this circumstance, the accreditation scheme would be a strong tool for quality assurance, however, we have only institutional certified evaluation and accreditation scheme by independent organizations certified by the MEXT as an accreditation organization. It requires 7-year cycle mandatory accreditation for all higher educational institutions based on a self-assessment report. From 2012, a research team supported by the MEXT grants studies and discusses a new scheme for professional (subject) evaluation for dental programs to enhance a role of accreditation in dental education. Following these movements, Japanese dental schools will provide dental programs that satisfy these elements to reach the goal to foster Japanese dentist, "Shikaishi" in the next few years.

Movement of professionals and Quality Assurance of professional education

Undergraduate dental curriculum in EU

"Quality Assurance" is a word for communication between the public and our dental society. The public means not only "Internal society" but also "Global society" in some cases. Across the European Union (EU), single social and economic area, mutual recognition of qualification and free movement of dentists are prescribed in EU Directive 2005/36/EC. License was basically for a country, an internal society, citizens and patients, however, qualified dentists from each member country can practice in other member country with their original license in the EU and EEA. It also affected undergraduate dental programs and the mobility of students for exchange programs. In 1999, ministers of Education of the European countries had signed Bologna Declaration to converge the higher educational systems. In dental education, the Association for Dental Education in Europa (ADEE), which has over 200 member schools throughout European countries, led many activities and projects called DentEd, DentEd Evolves, DentEd III to harmonize and converge dental education in European countries[2,3]. In 2009, the "Profile and Competences for the graduating European dentist—update 2009" was published to harmonize the curriculum in European dental schools as a result of a consultation on "Profile and Competencies for the European Dentist" published in 2005. It is consisted of 12 domains and each domain is supported by major competences and supporting competences[4].

ASEAN Mutual Recognition Arrangement on Dental Practitioner

The Association of Southeast Asian Nations (ASEAN) was established in 1967. ASEAN enhanced the cooperation among member countries to accelerate the economic growth, social progress and cultural development in the region. And member countries had agreed with the Mutual Recognition Arrangement to facilitate the movement of professional services in the ASEAN area. In professional services, the Economic Ministers at 14th ASEAN Summit in Thailand signed MRA on Dental Practitioner (MRA on DP), 2009[5]. The MRA on DP is discussed at the ASEAN Joint Coordinating Committee on Dental Practitioners (AJCCD). By the information of AJCCD, this MRA facilitates "the mobility of dental services professionals within ASEAN, enhance exchange of information and expertise on standards and qualifications, promote adoption of best practice for professional dental services and provide opportunities for capacity building and training of dental practitioners"[6]. Following this MRA on DP, the South East Association for Dental Education (SEAADE) has started to discuss their role for the MRA and has discussed core competencies in the workshop "Identifying Dental Professions Core Competencies of the SEAADE member countries" at the annual academic meeting in Bali, 2015. SEAADE will have some more discussion regarding this MRA. These movements will be affect on dental education in ASEAN countries in the next few years[7].

Movement of future dentists

People can move globally with the Free Trade Agreement and Economic Partnership Agreement. In other cases, a dentist can move to the other country if he/she fulfills some conditions to get a license in the country of destination. There is another way to assess graduates' competences by accrediting dental programs internationally. International accreditation and the global standard will be an important key to facilitate quality assurance in the context of global dental education. Dental educators and dental programs will cross the border in educational fields. In some cases, students cross the border.

The Commission on Dental Accreditation is an accreditation organization which accredits dental schools and programs in the U.S.. CODA has the Accreditation scheme and the Standard for Dental Education Programs for U.S.. And beside the U.S. accreditation scheme, CODA has a scheme for international accreditation[8]. There is a way to be a dental school accredited by CODA.

In Malaysia, The International Medical University (IMU), School of Dentistry has a special program, which provide international pathway for students and the program meets international standards[9]. The dental school has 4 partner dental schools, the University of Adelaide, The University of Queensland, the University of Otago and the University of Glasgow. In the IMU program, students study basic dental and medical sciences, introduction to the basic clinical skills at Phase I for the first 2.5 years. After that, students who choose partner dental schools will come to other countries to accomplish further 2~2.5 years and they will get respective degrees that are recognized internationally and locally by the Malaysian Dental Council. IMU designs their dental curriculum to meet international standards and share basic competencies of a Malaysian dentist with a dentist in other countries.

New accreditation Requirement in the U.S. medical education

International medical graduates who want to practice in the U.S. have to get a certification from the Educational Commission for Foreign Medical Graduate (ECFMG) to enter programs accredited by the

Accreditation Council for Graduate Medical Education (ACGME). ECFMG certification is a key for international medical graduate to get into the U.S. health care system. In 2010, ECFMG announced that only international medical graduates from accredited medical schools could apply for ECFMG certification[10]. This requirement will be in effective in 2023, however, those medical schools, which has not had the appropriate accreditation started to take a plan to change their curriculum to meet the accreditation. In response to this announcement, Japanese medical schools decided to establish the Japan Accreditation Council for Medical Education (JACME) in 2013 and will try to be recognized by the World Federation for Medical Education (WFME) as the accreditation organization in Japan, so that JACME is expected to have a role of accreditation organization internationally. In a plan, JACME will start the accreditation scheme for medical schools after 2017, and those medical schools that are accredited by JACME will be registered to the Foundation for Advancement of International Medical Education and Research (FAMER). WFME has a standard for medical education and this document is essential for accreditation of medical education[11]. Japanese medical schools followed the content of Model Core Curriculum for Medical Education however, the "Basic Medical Education, WFME Global Standards for Quality improvement", has been regarded as one of standards for medical curriculum and some of Japanese medical schools need to change their curriculum to enhance the clinical period and environment where students learn in real clinical procedures. It seems that it is not so important for those medical schools where their graduate don't intend to come to the U.S., on the other hand, this movement becomes a turning point for medical schools in Japan to assure the quality of medical education.

CONCLUSION

The relationships among countries are changing by the minute. To meet the global standard in dental education, we need to catch all information regarding dental education, especially about the accreditation. In this point, we need to look carefully not only undergraduate education but also Continuing Professional Development as postgraduate education. Establishing professional (subject) evaluation scheme is a key for internal quality assurance for the public, and also it will be a key for international recognition of Japanese Dentist "Shikaishi" in the world. "Shikaishi" and "Dentist", it is same meaning when we translate "Shikaishi" into English, however, Japanese dental educators have to reconsider our role for the society and the profile and competencies of "Shikaishi" for the coming future without translating it into English. "Dentist & Shikaishi", are they the same?

Conflicts of Interest

This investigation was supported in part by Health Labour Sciences Research Grant, Japan No. 201101008B

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Improvement and Enhancement of BioDental Education and Research in Hiroshima

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Key words: Globalization, BioDentistry, Dual linguistic education system, International Dental Course, Short-term programs

ABSTRACT

For more than a decade, Hiroshima University Faculty of Dentistry has been involved in the globalization of our education and research, driven by our perspective that the conventional dental education system would not be compliant to the imminent situation surrounding clinical dental care communities in a globalization era. On the other hand, we have a fundamental and strong belief that contemporary dental medicine must be grounded primarily on scientific knowledge consistent with modern biology, as represented by a new term "BioDentistry". These perspectives have been the driving force for the reformation that we made on dental education provided at our faculty. The most important challenge has been the use of English in our undergraduate dental program. Consequently this reformation has brought us with increasing opportunities to accept inbound international students staying in Hiroshima for 10 days to 4 years and send outbound Japanese students to sister universities around the world. We believe that these dynamic changes we exerted for the last few years have facilitated to cultivate global leaders playing an active part in worldwide dental medicine in future, extending the idea of BioDentistry.

COMMUNITIES FOR CLINICAL DENTAL CARE IN GLOBALIZATION ERA

"Globalization" is defined as a process through which people, money, information, and culture are interacted and integrated among different countries. Currently this process is a trend not only in the socio-economic world but also biomedical communities including those of dental medicine. This is well exemplified by the recent efforts made by ASEAN member states for their unification.

ASEAN countries are making steady and tremendous progress toward their economic unification by 2015. Associated with this reformation, mutual recognition agreement has been ratified to enhance the mobility of dental practitioners over the borders within the associated nations. Mutually recognizing dental practitioners requires a universal framework for dental education as well as healthcare systems. In fact, these countries are currently in discussions to establish standard competencies necessary for dentists who will be able to move abroad within the community.

We do not believe that such a revolution in the ASEAN community is just an affair of our neighbors. It may be supposed that dental medicine will necessarily be globalized in the near future over the entire Asian-Pacific area, including Japan. We can say with confidence that we cannot afford to be left out. The Trans-Pacific Partnership, an issue of current political argument, will also serve to accelerate mutual relationships in dental medicine as well in the course of economic unification. We should note that similar systems have already been laid out in the US/Canada, EU, and UK to accredit dental and dental-related education programs, so that dentists trained through compliant programs overseas can work as dental practitioners in these regions.

What would happen in our country as a result of further Asian globalization? This is a question that we have continuously asked by ourselves for the last decade. I guess a society will significantly change so that students and skilled labors such as dentists will be able to move more frequently within the Asian economic area, including Japan. In such a mobile society, the place of study and work will not matter any longer, and people will move in search for better environments and conditions. Consequently, the mobilization will make societies much more international and competitive. Accordingly we all will need to cooperate or compete with others in multinational classes, teams, and societies.

How can we prepare for the trend of the time in advance to survive as a dentist with global competencies? There is no doubt that improving our English skills is of primary importance because common language infrastructure is the basis for our international activities. However, I think for sure this is clearly not enough. More importantly, we need to precisely and deeply understand what is going on in other countries through direct experiences in a wide perspective, and then to develop new values and strategies, extending professional networks, for dynamically playing an active part in worldwide dental medicine.

The background mentioned above brought us with urgent concern that traditional dental education would

not been compliant any more for building dentists capable of surviving imminent global environments. Such perspective was our principal motivation for the drastic reformation that we have made in the last several years for the dental education system provided by the Faculty of Dentistry Hiroshima University. It should be noted that this revolution is quite coherent to our scope as a Super Global University (Hiroshima University has been selected as one of the Super Global Universities by the government under the national project aiming at intensively enhancing the globalization of Japanese universities). In addition, the Hiroshima Conference on Education and Science in Dentistry is one of the most important activities in which we have been involved for more than a decade toward globalization.

INITIATIVE IN BIODENTAL EDUCATION AND RESEARCH

"BioDentistry" is a coined word that we initially created almost a decade ago for representing the basic concept universal in scope of our dental education and research. The word BioDentistry denotes our strong belief that contemporary dental medicine must be grounded primarily on scientific knowledge consistent with modern biology.

We are quite sure that conventional dental education had exclusively stuck for long time to improve technical proficiency of dental students. As a matter of course, this is still important for warranting the quality of dental treatments, yet it is a time to reconsider that oral/maxillofacial malfunctions might be regarded as biological consequences, as the most of oral disorders such as caries and periodontitis are initially caused by bacterial infection. It should be stressed that we need to understand these conditions in the context of molecular and cellular biology. It is not until we logically understand the behaviors of bacteria and the responses of host defense systems that we are able to properly make strategies for prevention, diagnosis, treatments and improvement of prognosis. It seems that this aspect had been more or less overlooked in the conventional dental education.

Such a new paradigm in dental education facilitates to position dentistry as a part of contemporary biological sciences, which serves to provide multidisciplinary environments, activating scientific research in dentistry on an international level. This is demonstrated by the fact that most of recent progresses contributed by our research in Hiroshima, for instance, technological advances in regenerative dentistry and deeper pathological understanding of oral cancer, have always been made on the basis of current biological science and engineering.

Thus, initiatives for BioDental education and research are closely connected each other. We now know that such a circumstance in Hiroshima has great impacts on the manner of logical thinking by dental students as well as academic staffs. In addition, the advantage associated with BioDental education and research should benefit not only Japanese students but also international scholars. This is the reason why we strongly promote accepting international scholars as described below.

BIODENTISTRY LECTURES IN TRULY BORDERLESS CLASSROOMS

We are eager to promote students' competency to have a sense of purpose and behave more on their own initiative in global environments. We believe that one of the most effective ways to realize them is to expose students to really international circumstances.

Taking above into consideration, our School of Dentistry has just started simultaneous distribution of an international BioDentistry class using a teleconference system to the School of Dentistry at Airlangga University and University of Medicine and Pharmacy, Ho Chi Minh City. Distinguished lecturers from these three universities as well as University of Sheffield (in this case, 4 universities are connected each other) contribute to the program, sharing their own expertise from the viewpoint of both fundamental and applied aspects of advanced dentistry. Totally more than 230 undergraduate students at three classrooms in Japan, Indonesia, and Vietnam join this class all at once.

This provides an amazing opportunity for all students to extend their human networks, gaining deeper insights into cutting-edge BioDental research. Importantly, this highly international and interactive class makes students for sure aware of being the members of a contemporary Asian community.

DENTAL EDUCATION THROUGH ENGLISH

One of the most critical and important challenges we exerted ourselves during these years was the introduction of a new teaching method in 2012 to the dental education at the School of Dentistry, Hiroshima University. In the new method, English is thoroughly used in every class of lectures, exercises and practical trainings provided in the undergraduate program for 2nd - 5th grades.

English is obviously the language that is used in most international environments and therefore we believe that teaching dentistry through English serves for students to improve their communication skill, gain professional knowledge in English, and thus remarkably enhance students' international competency.

It should be noted that this is in fact a quite big challenge for Japanese schools not only in the field of dentistry but also any other disciplines, because education at Japanese universities has been traditionally and exclusively given in Japanese. Moreover, a linguistic problem is always a big issue for most of Japanese people and societies. Nevertheless, we have adopted the new teaching method to the education at the School of Dentistry for the first time in our country.

The reformation of a teaching method has required our great efforts. To optimize our education method, we have offered several faculty development workshops during years. In addition, we have promoted teaching staffs to take development programs for efficiently teaching in English, while individually consulting those who are not familiar with teaching in English. We have seen that all these efforts have served to gradually but certainly improve the quality of our education.

Another concern associated with this new method is quite specific to our country. Because our national board dental examination is done in Japanese, students are also

required to gain sufficient knowledge of dentistry with Japanese technical terms. That is why, even in the new teaching method, we need to teach students not only in English but also in Japanese. Therefore, we use both languages in every single class. Accordingly the system is called "dual linguistic education system (DLES)".

Our assessment after 4-year experiences with DLES tells us that the majority of Japanese students have gradually been getting used to the system, steadily gaining knowledge necessary for dental professionals. This is well evidenced by the successful result that we have seen in the recent CBT (computer-based testing conducted in Japan to assess if students' are sufficiently competent for starting clinical training at the university hospital) for the 5th-grade Japanese students who leaned dentistry for 4 years by means of DLES.

What is more important is that an increasing number of Japanese students are now interested in international activities. In fact, more than 40 Japanese students joined outbound programs during a year. We sent 2 students in a research track to University of Sheffield and Forsyth Institute. During last years many students participated in outstanding short-visit programs provided by University of British Colombia, University of Washington, Airlangga University, University of Medicine and Pharmacy at Ho Chi Minh City, Khon Kaen University, Taipei Medical University, Wonkwang University, and Pusan Catholic University. A dental camp in Cambodia, organized every year under collaboration with NGO Hiroshima, the Society to Protect the Teeth of Asian Children, also attracts an increase number of Japanese students.

ESTABLISHMENT OF INTERNATIONAL DENTAL COURSE

Thanks to the DLES, we are able to accept international undergraduate students all the time to the school of dentistry and invite them to our regular dentistry classes with approximately 50 Japanese students. This brought us with a great opportunity to create much international environments in a classroom, efficiently facilitating to cultivate international awareness of Japanese students as well as the international scholars.

The most obvious and significant outcome is that we have a possibility of inviting inbound students to be enrolled in the 4-year undergraduate dentistry program. Namely, in 2012 we established an International Dental Course (IDC), collaborating with our sister universities including Airlangga University, Indonesia, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam, and University of Health Sciences, Cambodia, with a tremendous support from the Japanese government as well as our alumni association. In the IDC program, we accept 3 students a year, each from the three schools. The students spend 4 years in Hiroshima to complete almost all the subjects necessary for reaching the level equivalent to a bachelor. At 4th to 5th grades, they also join one of the research laboratories to conduct cutting-edge BioDental research. Finally, credits granted to the students in Hiroshima are transferred based on our mutual accreditation.

After 4 years in Hiroshima, the students return to their mother countries and then start their clinical training at hospitals affiliated to their universities before licensed in their country as a dental practitioner. Actually, three IDC students of the 1st generation have just completed the program with great success and returned to their mother countries. We believe that they will act as global leaders in oral health communities in future. This year we have just selected three new IDC students of the 5th generation.

INCREASING CAPACITY FOR ACCEPTING INTERNATIONAL STUDENTS

The reformation we made on our education system has brought us with further opportunities: We became to be able to provide several new short-term stay programs for international inbound students from sister universities. The programs we are providing are available for undergraduate dental students, and the duration of their stay is ranging from 10 days to 1 year. These programs are also partially supported by the Japanese government.

In practice, we accept every year around 20 inbound students for the 10-day program. These students are able to gain many valuable experiences from the specially designed short-tem program that includes lectures, practical training, and clinical observation at the university hospital. Cultural excursions and intimate interactions with Japanese and other international students are also important elements of this program. Last year 18 students joined from Asian countries and one from UK.

In the 6-month (1 semester) program we normally accept 6 students every year. They are able to join a dentistry class at either grade from 2nd to 4th depending on the subjects they have already completed at their own universities. Similar to this, inbound students enrolled in the 1-year program (2 semesters) study dentistry for a year in either class with Japanese students. Last year we accepted totally 8 students from Indonesia, Vietnam, Thailand, Taiwan, and Brazil to the 6-month and 1-year programs.

Accordingly, a lot of international inbound students study at our school during a year. It is amazing to sometimes see that more than 20% of students in a single classroom are international inbound students. Such a quite international environment has been created in Hiroshima, most likely owing to the DLES.

CONCLUSIONS

As demonstrated in this paper, our globalization strategy is yielding a lot of success in cultivating future BioDentists in the world. We owe our success entirely to our sister universities and are obliged to the fact that we could conclude an academic exchange agreement with an increasing number of outstanding universities abroad.

It may be an indicative of our leadership in the globalization of dental education and research that we are committed to develop and implement globalized education programs for dentistry, dental technology, and dental hygiene under the national projects, such as "Program for Raising Standards of Dental Education Utilizing Integrative Functions, Program for Promoting Inter-University Collaborative Education (collaborating with Niigata University and Tohoku University)" and "Project for Developing a Program for Cultivating Global Dental

Care Professionals, Strategic Promotional Program for Cultivation of Core Professionals in Targeted Growth Fields", both driven by the Ministry of Education, Culture, Sports, Science and Technology.

What should we go further for taking a step toward more globalized BioDental education and research? We know for sure that one of the most important directions is to strategically strengthen our contribution to BioDental research through functional enhancement at the postgraduate level. Apparently our efforts to acquire a continuous and sufficient budget are critical for further progress in globalized BioDental education and research.

Session VII

Food, Nutrition and Health

Which Foods are Suitable to Elderly Dysphagia Person?
—According to Japanese Criteria of Dysphagia Foods—

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Which Foods are Suitable to Elderly Dysphagia Person? —According to Japanese Criteria of Dysphagia Foods—

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ABSTRACT

Eating provides great pleasure for elderly people. For those with dysphagia, it is necessary to provide diets in the form that is suited to their level of chawing and swallowing function.

However, as each hospital established dysphagia diet, it cannot correspond to acute hospital and other hospital. Therefore some dysphagia patient occur the treble such as phenomena. To prevent these trebles, several standardized stages have been developed in order to promote smooth interhospital cooperation. In this paper, we introduce three criteria of dysphagia food.

INTRODUCTION

In Japan, as the number of elderly persons has increased remarkably, so has the number of those with dysphagia. There are several causes of dysphagia; in acute care hospitals, dysphagia is commonly caused by stroke1). In the Japanese medical system, seriously patients are hospitalized in an acute care hospital for approximately 2 weeks and are then transferred to a chronic care or rehabilitation hospital.

Eating also leads to direct training for persons with dysphagia. In many hospitals, gradually modified diets are provided for dysphagic patients, utilizing creativity; as such, diets come in several forms that can vary depending on the hospital.

However, as each hospital established dysphagia diet, it cannot correspond to acute hospital and other hospital. To arrange dysphagia food, there are some criteria for dysphagia food.

Classification of dysphagia diets

1. Dysphagia diet pyramid

A gradual dysphagia diet for acute care hospitals was presented by Seirei Mikatahara General Hospital. Since the late 1980s, the number of patients with dysphagia has gradually increased, and the five-phase dysphagia diet staging system was developed with clinical trial. This staging system is called the "dysphagia diet pyramid" (Figure 1). In the dysphagia diet pyramid, each stage of hardness, adhesiveness, and cohesiveness was established by Sakai et al. in 2006 (Table 1)2). Table 1 is mainly used for patients with pseudobulbar palsy. Currently, it is used as reference material for those with dysphagia following modified diets in many acute care hospitals. Cookbooks with recipes for each stage are now available, and books regarding the classification of commercial foods are also available.

For dysphagia due to stroke, jelly type foods in sliced form are suitable and easy to chew compared with the paste form. Therefore, the five-phase dysphagia diet staging system ranges from jelly type foods at Level 0 to soft foods at Level 4 (for patients with severe and mild dysphagia, respectively).

The forms of foods at each stage are as follows:

Level 0 (L0): no protein jelly such as green tea jelly or fruit juice jelly (swallowing training foods for persons with severe dysphagia):

L1: mousse form foods (except protein-rich meats and

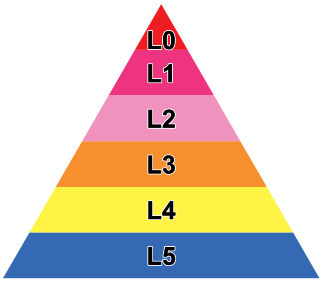

Figure 1. Dysphagia diet pyramid

Table1. physical property of dysphagia diet pyramid

	L0	L1	L2	L3	L4
Hardness (N/m^2)	2000-7000	1000-10000	Under12000	Under15000	Under40000
Cohesiveness	0.2-0.5	0.2-0.7	0.2-0.7	0.2-0.9	0-1
Adhesiveness (J/m^3)	Under200	Under200 (In case of cohesiveness near 0,4, Under500)	Under300 (In case of cohesiveness near 0,4, Under800)	Under1000	Under1000

Table 2. Food for special dietary use; authorization standards of food for persons with dysphagia

	Standard 1	Standard II	Standard III
Hardness ($\times 10^3 N/m^2$)	2.5-10	1-15	0.3-20
Adhesiveness (J/m^3)	Under400	Under1000	Under1500
Cohesiveness	0.2-0.6	0.2-0.9	—
	Homogeneous like jelly	Homogeneous like jelly and mousse	Homogeneous and Heterogenous like unison rice porridge and soft paste

fishes)
L2: mousse form foods (including meats and fishes)
L3: paste or pureed food
L4: soft foods; several formed foods are also included
L5: general diet

The method of measurement of the physical properties of food is as follows: foods to be measured are placed on a petri dish 40 mm in diameter and 15 mm in height; using a creep meter, the food was compressed twice at a rate of 1 mm/s. Based on the obtained curve, factors such as hardness, adhesiveness, and cohesiveness are calculated.

2. Food for special dietary uses (FOSDU); authorization standards of dysphagia diet

In 2009, the Ministry of Health, Labour and Welfare reviewed the food for special dietary use; that is, food that requires permission by the authorities to be able to display that the food is appropriate for special dietary uses (e.g., for infants, children, pregnant women and nursing mothers, and patients with dysphagia). The standards for food for persons with dysphagia were established following this review, and are developed based on the dysphagia diet pyramid, which means that they bear resemblance to those for the dysphagia diet pyramid evaluation criteria (three items such as hardness, adhesion, and cohesiveness are used). However, the values are different, since the method of measuring physical properties is slightly different from that of the dysphagia diet pyramid (Table 2).

The method of measurement of the physical properties of food is as follows: foods to be measured are placed on a petri dish 40 mm in diameter and 15 mm in height; using a creep meter, the food was compressed twice at a rate of 10 mm/s. Based on the obtained curve, factors such as hardness, adhesion, and cohesiveness are calculated.

3. Dysphagia Diet 2013 by the JSDR dysphagia diet committee (JSDR2013)

The Japanese Society of Dysphagia Rehabilitation (JSDR: consisting of approximately 11,000 members) created a special committee to address the modifications made to the diet of those with dysphagia. This committee developed and presented the 2013 classification of a modified diet for dysphagia persons (JSDR2013) (Figure 2). The main purpose of developing the JSDR2013 was to promote common understanding of modified diets for dysphagia patients in clinical settings. This classification was considered for not only acute care hospitals, but also chronic care hospitals and welfare institutions for the elderly. There is also a correspondence table of the JSDR2013 to compare with the dysphagia diet pyramid, food for persons with dysphagia as food for special dietary use, and other classifications (Table 3). One topic of note that is mentioned in the JSDR2013 is the classification of thickness/viscosity as well as diet.

Table 3. Correspondence Table at JSDR2013 and the dysphagia diet pyramid or Food of special dietary use (FOSDU)

Code	Dysphagia Diet Pyramid	FOSDU
0j	L0	I
0t	A part of L3	
1j	L1+L2	II
2-1	L3	III
2-2		
3	L4	
4	L4	

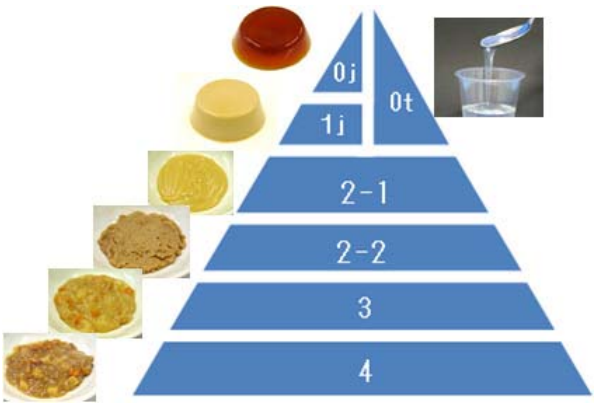

Figure 2. Diagram of the 2013 classification of a modified diet for dysphagic persons

Code 0 foods are those in the form of protein-free jelly or thick water used for swallowing training. Protein is removed under the consideration of inflammatory responses due to aspiration pneumonia. The jelly foods in sliced form can be easy swallowed, even if the tongue and the oral cavities are immobile.

Code 0j foods include those that are homogeneous and less syneresis, These are equivalent to the foods at L0 of the dysphagia diet pyramid.

Code 0t refers to thick liquid, including thickened tea and orange juice as protein-free liquids; the degree of thickness corresponds to "Moderately thick" or "Extremely thick" of the JDD2013 (thickness).

Code 1 foods include jelly-, pudding-, or mousse-form foods for dietary intake; they are homogeneous, and factors of adhesion, cohesiveness, hardness, and syneresis are taken into consideration.

Code 2 foods are purees, pastes, and blended foods. They have a fluid quality and are smooth, not sticky, and easily mixed; in spite of this fluidity, Code 2 foods are not fluids that can be passed through a tube. They can basically be eaten using a spoon, forming a bolus of food in the mouth by simple operation; consideration is given for neither residues nor incorrect swallowing at the pharynx. According to homogeneity, Code 2 is subdivided into homogeneous 2-1 and heterogeneous 2-2. Even if the food is categorized as heterogeneous, it should be soft enough that the drop can simply be swallowed without needing to be crushed by the tongue.

Code 3 foods are formed-foods that can be crushed easily by the tongue and palate; a bolus of food can be formed and transferred easily, with consideration to avoid being scattered. There is not a lot of syneresis caused during chewing and transferring in the oral cavity.

Code 4 foods are formed-foods that can be easily cut using chopsticks and a spoon, but some mandatory force is required because such foods are difficult to crush using the tongue and palate; after chewing, these foods are easily gathered up as a bolus without breaking up the components.

SUMMARY

We introduce Japanese dysphagia diet categories such as Dysphagia diet, FOSDU and JSDR2013. The jelly type food located most easy for dysphagia patient in these categories. Main cause of dysphagia in acute hospital is pseudobulbar paralysis of stroke. The jelly type food is suitable compare to puree type food in this case. The jelly is not necessary to chewing and that is not spread in the mouse. Medical stuffs serve the jelly to make slice type and go into mouse in patient.

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Nutrition, Food and Dietetics for Health

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1. Diets of human

Homo sapiens who was our ancestors got the curiosity and creativity by making development the cerebrum depend on the evolution of bipedalism. By that, we got the omnivorous enough not found in other animals to distinguish between food and poison by anything eat experience in the severe environmental changes. The diversity of diet and the stability of the food supply further increase by the development of agriculture and cooking.

However, animals and plants which were selected do not exist intended to be the original food for human. Individual food supply energy and nutrients to partially to human, but not intend to us fully guarantee the human health by supplying the necessary amount of all nutrients. We can not live to eat only bamboo leaves like the panda.

In other words, human evolved omnivorous to secure the required amount of energy and all the nutrients in the diet which is a combination of food to complement the problems of individual foods. The human can live everywhere on the earth by such the way, and then the agricultural development has enabled an increase in the population. Furthermore, since not using more energy in the digestion as herbivores, it was possible to develop the cerebrum.

However, in order to maintain the omnivorous, the ingenuity and wisdom to be able to properly select and to eat an appropriate amount from a number of foods became necessary. Nutrition science was born and developed as a method of obtaining the scientific basis. So human experienced the food shortages by disaster and war and inadequate food selection by the eating habits many times.

In other words, human have been saddled the fate of "eat to think nutrition" by the omnivorous.

For example in Japan, there were high infant mortality rates and infectious diseases such as tuberculoss by malnutrition which was caused with modest contents of the rice the staple food.

By malnutrition to overlap excessive intake of salt, high blood pressure, stroke, and even people who die of stomach cancer were many. After all, the Japanese was short-lived. The low nutritional meal with traditional Japanese and post-war food shortages of the pre-war with the introduction and nutrition education of post-war Western diet, the current excellent Japanese food that balanced nutrition is formed.

2. Over nutrition and Under nutrition are mixed

Japanese diets is a balance of nutrition on average and good for health. But in recent years, what is discussed, it is the extension of healthy life expectancy rather than the average life span. To prolong the healthy life expectancy, it is necessary to prevent lifestyle diseases and senile syndrome. Lifestyle related disease like diabetes mellitus, arteriosclerosis, ischemic heart disease, for the prevention of stroke, visceral fat made with these risk factors, hyperglycemia, lipids, and improvement of lifestyle to reduce blood pressure, etc. and is the target.

Prevention point of one of the geriatric syndrome is "flail". The Flail, by a decrease in various functions decline and spare capacity associated with aging, it refers to a state in which increasing vulnerable with respect to disease onset and physical dysfunction. First cause of falling to flail is to fall into the low nutritional status.

If the amount is gradually decreased continued shortage state of energy and protein intake, it becomes sarcopenia, activities force, decreased muscle strength, and physical function, energy consumption is reduced, as a result, appetite is reduced, furthermore intake is reduced, if malnutrition is Kore with serious flail is formed.

So the present nutritional problem in Japan is that the excess nutrients with lifestyle related diseases and malnutrition are mixed.

3. Healthy diet and how to eat

In "Japanese Dietary Reference Intakes 2015 edition" recommended amount and recommended dose to avoid from the nutrient deficiency, tolerable upper intake to avoid from the nutrient excess diseases, target amount has been set to avoid risk of lifestyle-related diseases. In other words, nutrition is to clarify the proper intake of energy and nutrients needed in a day, by the "Food Guide", these values in terms of the intake of foods and food groups, after all, what eat habitually on the average have been solved almost.

However, in the 21st century, there is excess nutrition and malnutrition are mixed "Double burden of malnutrition DBM", nutrition problem is becoming diversified and complexity. In addition, the biological rhythm from the viewpoint of environmental adaptation, "How to eat" it is becoming a challenge in recent years.

The authors have cleared that diet induced thermogenesis (DIT) decrease on revealed the by skipping breakfast on the evening type meal in the same nutritional content of the day. In addition, DIT varies with the speed to eat meal time, furthermore due to the difference of the combination of order and food intake, metabolism of energy and nutrients has been observed to change.

The ministry, in June 2013, launched the "Study Group on the way of" Healthy Eating "to support the longevity of the Japanese." Its purpose is to make the concept organizing of a healthy diet, and create a mechanism to ensure the standards and their quality based on a

scientific basis, to promote health maintenance and disease prevention, and creating the associated health industry, a "health it was to promote the development of meal" to practice a friendly environment.

About the results of the study from the various fields of up to one year, after all, it has been the definition that the "Healthy Diets" mean "the state of which is based on a nutritional balance that is required for maintenance and promotion of a healthy mind and body is sustainable without difficulty".

Then, it is considered necessary the following conditions in order to achieve a healthy diet.

1) along with the take over the goodness of Japanese food culture, it is important to be accompanied by a delicious and fun. Taste and fun, ingenuity of ingredients and cooking, the formation of the food tastes and diet views, such as the selection of the food scene, and a wide range of elements.
2) people, makes it possible to routinely access to appropriate food social, economic and cultural conditions are in place.
3) it has been carried out initiatives such as education and experience activities related to food of stable supply of secure and diet that takes advantage of local characteristics.

We do not ingest nutrients itself directly, but cook foods, a menu that is a combination of food is ingested at a certain eating. In other words, maintain a healthy state, and a healthy diet that can improve, nutrients intake fall in the range that has been set from the Dietary Reference Intakes, the best and the possible food and cooking taking of Japanese, suitable for biological and environmental it is necessary to be consumed in the process.

This process is constructed from a wide range of components.

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Impact of the Gut Microbiota and Probiotics on Health

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Key words: microbiota, gastrointestinal tract, oral cavity, probiotics, *Lactobacillus casei* Shirota, lactobacilli, bifidobacteria.

ABSTRACT

An adult human gastrointestinal tract is inhabited by up to 10^{14} indigenous bacteria. These bacteria belong to over 1,000 different species and interact with each other as well as with their host (Qin et al, 2010). The gut microbiota plays an important role in human health by providing a barrier against a colonization of pathogens. It is accomplished by stimulating the development of the immune system and by utilizing indigestible fiber to produce short-chain fatty acids that act as energy substrates for the host (Guarner and Malagelada, 2003). A human oral cavity also has microbiota which is composed of over 700 different species (Aas et al, 2005) and its disturbance causes oral disorders such as periodontal disease and dental caries (Do et al, 2013).

Probiotics are defined as living microorganisms that confer a health benefit on the host when administered in adequate amounts (FAO/WHO, 2002). *Lactobacillus casei* Shirota (LcS) is a well-known probiotic strain and it induces health benefits such as improvement of bowel function (Sakai et al, 2015) and reduction of infection incidence (Gleeson et al, 2011).

During my talk, I will briefly summarize the gut/oral microbiota and introduce the properties of probiotics by showing the data about LcS. Our recent findings with regard to the probiotic candidates in human oral bacteria for the prevention of dental disease will also be presented.

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Session VIII

International Collaborative Program for Human Resources in Dental Medicine at Graduate-level

How are We Producing the Next Generation of Dental Faculty Members?

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ABSTRACT

University research is critical to pushing the envelope of knowledge forward to enable improvements in patient care to occur. Dental schools/Faculties are the critical element to generate new knowledge for oral health care. Faculty members academic roles include accomplishment of scholarly activities so it is critical that we have individuals with strong research preparation and novel research ideas. There is now a shortage of dental faculty members and in the United States there may be as many as 400 faculty positions that remain unfilled. Often the reason that no new faculty member was hired is due to a lack of candidates with the appropriate experience to complete research, teaching and service at the levels expected by the university. This situation is not limited to the US but also occurs in other countries, including Canada. The unfilled faculty slots have been reported by numerous authors and a key factor for a position remaining unfilled is that the applicants do not have sufficient preparation to complete original scholarship. The pipeline for these new faculty members has traditionally been dental school graduate programs that combine clinical specialty training with original research projects. However, the numbers of students choosing an educational program that combines advanced dental specialty and fundamental research training has dwindled. There are multiple reasons to explain the decline in graduate education in dentistry. In some cases this may be the result of the large amount of educational debt that dental students accrue to complete their basic dental degree. In other cases it may be that the income of dentists in private practice is much higher than the salaries of dental faculty members. There are other instances where the inability to secure grant funding to support research leads a new faculty member away from an academic career. The result is fewer dentists receiving the type of educational experience that would result in strong preparation for a faculty position. The consequence is that dental research has fewer scientists pursuing questions that could ultimately improve the oral health of patients. One solution is that dental schools must encourage their brightest students to consider an academic career and develop programs that mentor their career development to insure that there is another generation of scholars entering dental academics. It is a critical time in the development of the next generation of dental scientists.

A brief History of Dentistry as an Academic Discipline in Universities

The Gies Report in 1926 provided a foundation for examining dental curricula at schools in the United States and Canada[1]. The results of that report led to dental schools increasingly becoming integrated with major universities, which led to dental schools adopting the research, teaching and service cultures of their universities. This was a major step forward in dentistry becoming a learned profession. One major responsibility of any dental school should be a commitment to research producing the outcomes that will lead the profession forward. Gies made a prophetic quote in 1926 when he said, "Each dental school should encourage and support research by its teachers and should arouse and develop creative capacity and professional leadership among its most gifted students." Dental faculty members did become more active in research and several major findings helped to improve oral health. The importance of oral health research was recognized by the US Congress in 1948 when the National Institute of Dental Research was founded as one of the six original institutes[2]. The NIDR quickly became one of the primary supporters of dental research and the research initiatives in dental schools grew rapidly and became aligned with their parent universities. As the quantity of dental research expanded it was necessary to have increasing numbers of faculty members qualified to conduct this research at the highest levels. Dentist-scientists and PhD-scientists collaborated to grow the research capacity of dental schools. In the 1960's until the 1980's there was increasing support for dental research, training grants to produce the next generation of scientists and a commitment by dental schools to a robust research program. Unfortunately the research support began to diminish due to fiscal constraints in government and the investment in oral health research declined. In 1995 the Institute of Medicine of the US National Academy of Science again reviewed dental education, in ways similar to those Gies followed in 1926. Their report was published, *Dental Education at the Crossroads: Challenges and Change*[3], and the conclusions were of interest both in comparison to the Gies report and to the status of dentistry as a learned profession. Specific recommendations related to the research mission of dental schools included #9, "To expand oral health knowledge and to affirm the importance of research and scholarship," #10 "all dental schools should develop and pursue collaborative research strategies that start with the academic health center or the university and extend to industry, government, dental societies and other insti-

tutions able to support or assist basic science, clinical or health services research," and #11 "to strengthen the research capacity of dental schools and faculty." It was recognized that it would be critical for academic dentistry to prepare new scientists who would emphasize oral health problems in their research. We are now 20 years after the IOM report and still discussing ways to achieve the recommendations generated by that report. One current major deficiency is the number of dental faculty members prepared to conduct scientific investigations at the highest levels.

A deficiency in the number of academic dental faculty members

There have been many publications reporting the number of faculty positions open at US dental schools and the reasons that these positions have not been filled[4-12]. It has been reported that there may be as many as 400 faculty positions that are open in US dental schools. As the number of US dental schools has grown the lack of academically prepared faculty members has grown. There have been many reasons used to explain the open positions, which include expectations for the faculty member that exceed their education/experience, expectations for promotion and tenure that exceed achievement, faculty salaries that are lower that clinicians in the private sector and a diminishing pool of grant funding to support research programs[7,8,9,10]. The result of these obstacles for faculty members is that dental students learn that an academic career is difficult, time-intensive and not economically rewarding. So some of the best students with potential to be outstanding faculty members are being led away from academic careers. So if our best and brightest students are not being encouraged to follow a career development pathway that would prepare them for success as an academic faculty member then where will we find the faculty members that are needed? Currently, many US and Canadian schools are dependent on immigration to fill open positions, bringing dental educators and dental scientists from international locations[5]. The other pool of new faculty members is coming from dental private practice, dentists retiring from practice and becoming faculty members[4]. Is this a sustainable model? If outstanding people are leaving other countries for US positions, what happens to the faculty profile in the dental schools in their home countries? If a person leaves private practice after 25 years to become a faculty member what preparation do they have to complete a full academic profile of research, teaching and service? What are our current dental students learning about potential career opportunities as a dental faculty member? The best sustainable approach to maintaining a robust dental faculty pool is to follow the recommendation that Gies made in 1926, "arouse and develop creative capacity and professional leadership among its most gifted students." The lack of a commitment of dental students to an academic career has resulted in the inability to fill open faculty positions.

Approaches to address the shortage of faculty members

Currently the majority of new faculty members appointed in dental schools are coming from private practice. These individuals are most often appointed in clinical stream faculty positions since their career profile would not be consistent with a tenure stream appointment[12]. While these hirings fill positions and bring in clinicians able to teach in the clinical disciplines it does not address the deficiency in dental scholars who would conduct the research necessary to push the profession forward. Since many of these individuals coming from private practice have already had a 20-25 year career they will be available as faculty members for a relatively short period of time and it is unlikely that they will add a career development pathway linked to research. Some may pursue additional education in education-related areas that will benefit the dental school but not research productivity. There could be some scholarship of teaching and learning (SOTL) productivity if the environment supported it. There is the potential to engage these individuals in clinical research projects that utilize their expertise in patient care, however these projects will likely need to be developed and funding obtained by full time tenure stream faculty members. It will be critical to balance dental school faculty hiring to insure both the capacity to deliver the dental curriculum and to engage in cutting edge dental research.

Another approach to dental school hiring for faculty members to engage in research is to recruit productive faculty members from another dental school[9]. This will increase the quantity of research occurring in the "recruiting" school but decreases the amount of research at the "recruited from" dental school. The result is no gain overall in the amount of dental research and likely an overall increase in costs (salary, start-up, etc) thus an actual decrease in the research return on investment. This may provide short term benefits for the "recruiting" school but this is not a sustainable process. If the person who is recruited is a "builder" or "magnet" then the culture at the recruiting school may change and a new cohort of trainees develop based on the research profile of the recruited faculty member. A new cohort of trainees is the most productive approach to generate additional dental scientists.

New dental scientists could come from PhD graduates of basic science programs recruited to do research in dental schools, however these individuals may not be well versed in the types of problems that exist in dentistry; diagnosis, treatment, management, prevention. New young research-focused dental faculty members should optimally come from dental school graduate education programs. Importantly, linking a dental specialty with the research training would be optimal since dental schools tend to recruit faculty members into the specialty disciplines. These individuals would also be quite familiar with the clinical problems that need to be investigated at the basic biomedical level that could eventually lead to translation into new therapeutic approaches. This would require dental graduate programs to emphasize both dental specialty clinical education as well as a strong graduate research education. There are many programs world-wide that have such an educational profile however there appears to be a decline in the numbers of graduate students interested in this type of career development, which is focused on a dental academic position. It

is critical for dental faculty members to mentor dental students who are inclined towards an academic career and model the best practices of a faculty member. A culture where faculty members complain about being underpaid, overworked and underappreciated is not one that will incubate the interest of a dental student in an academic career. Mentorship is the critical component and that requires dental faculty members with the career profile that is desired to reach out to the inclined dental students and continue to nurture their interest and provide guidance into educational pathways that will be optimal preparation for a faculty career.

Changes that could increase the numbers of potential faculty members

In order to increase the pipeline of potential dental school faculty members several things will need to be done. It may not be possible for all of them to be done at every dental school but if all dental schools collectively can achieve these goals then there should be more potential new faculty members for all the dental schools.

1. Encourage dental students to include research in their preparation
   Many dental schools have student research groups that provide a forum for students interested in research to pursue that interest. It is important that the students commit to an in depth research experience and not have as a goal to answer "Yes" to a graduate admissions question, "do you have any research experience?" Dental student research should be suitable for publication and the role of a dental student in published research clearly identified. Faculty members with a research-intensive profile should be encouraged to participate with these students and engage their interest in research as a potential career profile.
2. Develop DDS/DMD- PhD curricula
   The PhD is the entry level degree for scientists in all other biomedical departments and to compete on an even footing with scientists in other disciplines it will be important for dental-scientists to have the preferred entry level preparation. These combined programs will need to integrate the clinical and research curricula to avoid duplication of learning and to provide the student with the incentive to pursue two degrees. There will also likely be a need to provide some tuition support to avoid dental school debt from becoming an impediment to an academic career. The graduates of these programs should also be encouraged to continue their research preparation with a postdoctoral research position, which is also typical of research faculty members in other departments.
3. Develop dental specialty-postdoctoral research fellowship programs
   For an individual with a DDS/DMD-PhD preparation a postdoctoral research experience is critical to their success as a scientist. Additionally dental schools typically recruit faculty members into discipline specific departments. Thus a successful faculty recruit would need both a dental specialty and a postdoctoral research experience. For an individual with a PhD the postdoctoral research experience could substitute for the graduate research education currently linked to dental specialty graduate programs. A person completing this type of program would be very well prepared for success as a faculty member.
4. Mentoring programs for new junior faculty members
   A new faculty member has considerable preparation for their clinical discipline and their area of research however they have little preparation for negotiating the academic pathway of a tenure-track faculty member[12]. This preparation can be accomplished through a formal mentoring program by more senior faculty members who have demonstrated success in their academic career. These mentoring programs should provide assistance in research grant preparation/management, introductions to those individuals with important roles in research both on campus and with national funding agencies, clearly defined goals for the accomplishments necessary to achieve promotion and tenure, and defined protected time to achieve their research objectives. There should be oversight from department heads and school administration to insure that the mentoring occurs and achieves the intended outcomes.
5. Writing programs for junior faculty members
   One area that most new faculty members have had limited experience is scientific writing. This type of writing is essential to prepare manuscripts for publication, grants to funding agencies and communications with collaborators. Most often this is an area that is not featured in graduate education and many PhD mentors provide a major editing role in documents but not a way to enhance the scientific writing abilities of trainees. A formal program with a skilled professional in scientific writing could enhance success with grant applications and facilitate publication of scientific findings. Both of these are critical to the success of a junior faculty member and developing a program to help achieve that success would be a significant resource.

CONCLUSION

There is clearly a lack of individuals optimally prepared for a successful career as a dental academic. Current strategies have provided some short term answers to address the problem. However a sustainable long term solution is necessary to insure that the profession of dentistry continues to achieve the scholarly objectives. More people are needed in the dental faculty member pipeline and it is essential for dental educators/administrators to develop the programs to address this requirement.

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Clinical Academic Training for the Next Generation of Clinician Scientists: The UK Experience

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Key words: Dentistry, Clinician Scientist, Postgraduate training, United Kingdom, National Health Service, Health Education England, NIHR

ABSTRACT

Postgraduate training for clinician scientists in the UK is complex and is overseen by multiple organisations. To become a senior clinical academic, working in a University and undertaking research and teaching a dentist must train for up to 12 years after qualifying as a dentist. They must meet the requirements of the National Health Service to become a specialist dentist AND they must undertake a research training and obtain a PhD.

INTRODUCTION

In the United Kingdom postgraduate training in clinical specialties is quite complex and is overseen by multiple agencies. The vast majority of clinical care is delivered by the National Health Service (NHS) and all clinical training is undertaken in NHS hospitals or clinics. To be a specialist dental clinician, you must train in the NHS and pass examinations set by the Surgical Royal Colleges, and then be registered by the General Dental Council. The Universities train undergraduate dentists (Speight & Farthing 2013), but have very little role in the clinical training of postgraduate dentists, and very few postgraduate degrees are recognised for accreditation as a specialist clinician. However, to become a clinical academic or a clinician scientist, it is expected that a dentist will have research training and will have studied in a University for a PhD. In reality therefore, the University Dental Schools and the NHS Dental Hospitals work closely together to cooperate in training.

DENTAL SPECIALTIES IN THE UK

There are 13 recognised dental specialties in the UK (Table 1), which require between 3 and 5 years of training. A three year training period will allow a dentist to register as a specialist and practice their specialty in their own private clinics, usually under contract to the NHS. For other specialties a 4 or 5 year training programme is required to enable the specialist to work in an NHS hospital as a Consultant - the highest rank available to a doctor or dentist in the NHS. In some specialties (eg. Paediatric Dentistry and Orthodontics) a dentist can undertake 3 years training to register as a specialist and then a further 2 years to be eligible for a consultant appointment. To be able to call oneself a specialist a dentist must have satisfied all the training requirements and be registered as a specialist by the General Dental Council.

Table 1. There are 13 recognised dental specialties in the United Kingdom.

Dental Specialty	Duration of training (years)	Number of dentists in UK
Dental and Maxillofacial Radiology*	4	25
Endodontics	3	115
Dental Public Health	4	258
Oral and Maxillofacial Pathology*	5	31
Oral Medicine	3-5	69
Oral Microbiology	5	7
Oral Surgery	3	735
Orthodontics	3 (+2)	1349
Paediatric Dentistry	3 (+2)	239
Periodontics	3	346
Prosthodontics	3	430
Restorative Dentistry*	5	308
Special Care Dentistry	3	316

The training period is from 3 to 5 years. Those marked * are restricted to NHS hospitals only. The other specialties may be practiced in primary care dental clinics. For some a period of further training (+2) will make the specialist eligible to practice in an NHS hospital (Data from GDC).

THE TRAINING ENVIRONMENT

Postgraduate training is overseen or regulated by a number of agencies, which cooperate and interact to provide a properly trained workforce (Table 2). England, Scotland, Wales and Northern Ireland have slightly different structures but the situation in England will be described for ease of understanding.

General Dental Council

The dental profession is regulated overall by the UK government by an Act of Parliament called the Dentists Act 1984 (Dentists Act, 1984). This act sets the rules and laws as to how the profession should be regulated and

Table 2. A summary of the main organisations who oversee, regulate or provide postgraduate education and training in the UK

Organisation	Agencies	Role
General Dental Council (GDC)		Regulate the whole dental profession in the UK and hold the register of all dentists and dental specialists. Oversee and approve the curricula for UG and postgraduate training. All training in the UK is therefore quality assured to a single national standard.
National Health Service (NHS)	Health Education England (HEE)	Commissions and funds all health education and oversees workforce planning
	Local Education and Training Boards (LETBs)	13 regional committees of HEE which organise training and education of all NHS staff
	Dental Postgraduate Deans (PGD)	Each LETB has a DPGD who is responsible for the PG education and training of dentists in their area
	National Institute for Health Research (NIHR)	NIHR is the research branch of the NHS and is responsible for commissioning and funding clinical research. They also fund research training for clinician scientists
	NHS hospitals and clinics	These provide the vast majority of health care in the UK (90%+). They also received funds from HEE and from NIHR to support education and training and research. All specialist dentists in the UK are trained in NHS hospitals or clinics
Royal Colleges of Surgeons		The colleges provide all the examinations for the specialty qualifications. The GDC regulate the examinations and recognise them for specialty registration. Some specialties have separate colleges, eg. Royal College of Pathologists.
Universities	Dental Schools	Independent educational establishments. In Dentistry the Universities train UG students in cooperation with their NHS Dental Hospital. Universities provide postgraduate research degrees (PhDs) and Master's qualifications.

who can or cannot practice dentistry. The implementation and policing of the act is delegated by the Government to the General Dental Council (GDC) who are responsible for regulating the whole of the dental profession. The role of the GDC in regulating undergraduate training in UK Dental Schools has been described previously (Speight & Farthing, 2013). However, the GDC also regulate all postgraduate training and therefore ensure a single national standard for quality assurance across all schools and hospitals. All postgraduate trainers must meet minimum standards and all specialists must be trained according to an agreed national curriculum. The GDC is advised by experts in each specialty in the form of *Specialty Advisory Committees*, who advise on the content of the curriculum and on the appropriate assessment methods. The GDC holds the national register of specialist dentists.

National Health Service

The NHS is a very large organisation and is one of the largest employers in the world. It provides virtually all the routine healthcare to the whole population of the UK and is funded largely through taxation. The NHS is overseen by the Department of Health (DH), a government ministry. Through its various agencies it also funds clinical research, and education and training for its workforce. For undergraduate training of dentists (and doctors) the NHS funds the clinical aspects of the training programme, while the academic training is funded in part by the student (through fees) and in part by the Higher Education Funding Councils. These also derive their income through taxation but are overseen by a separate government ministry, the Department for Business Innovation and Skills (BIS). The NHS provides almost all the postgraduate specialist clinical training for both dentists and doctors. They provide the clinical working environment (in NHS hospitals and clinics) as well as the salaries of the trainees. Because the NHS funds this postgraduate training, the overall number of specialists who are trained and allowed to sit the qualifying exams are regulated and restricted to meet the needs of the workforce. Without an NHS funded post, it is very difficult to obtain a specialist clinical training in the UK.

Health Education England

HEE was formed only about 5 years ago as the organisation with overall responsibility for the education, training and development of all staff in the NHS. They monitor the workforce and commission education and training from a number of different agencies. In the case of Dentistry, they commission and fund undergraduate education from the Universities and postgraduate education from NHS hospitals and from private providers of continuing education programmes.

HEE is divided into 13 regional committees called *Local Education and Training Boards* (LETB), which are responsible for delivering education and training within their region. Each LETB will employ a *Dental Postgraduate Dean* (DPGD) who is the person responsible for looking after all the postgraduate dental trainees within his or her region.

Since 1993, all newly registered dentists who have qualified in the UK must undertake one year of supervised postgraduate clinical training called *Dental Foundation training*. This is undertaken in general dental practices and is supervised by experienced dentists in their own practice. This programme is funded by HEE and is managed by the DPGD. The DPGD also manages all the dentists who are training to become a specialist.

National Institute for Health Research

NIHR is essentially the research and development branch of the NHS. It provides funds for clinical research and for the research training of the NHS workforce.

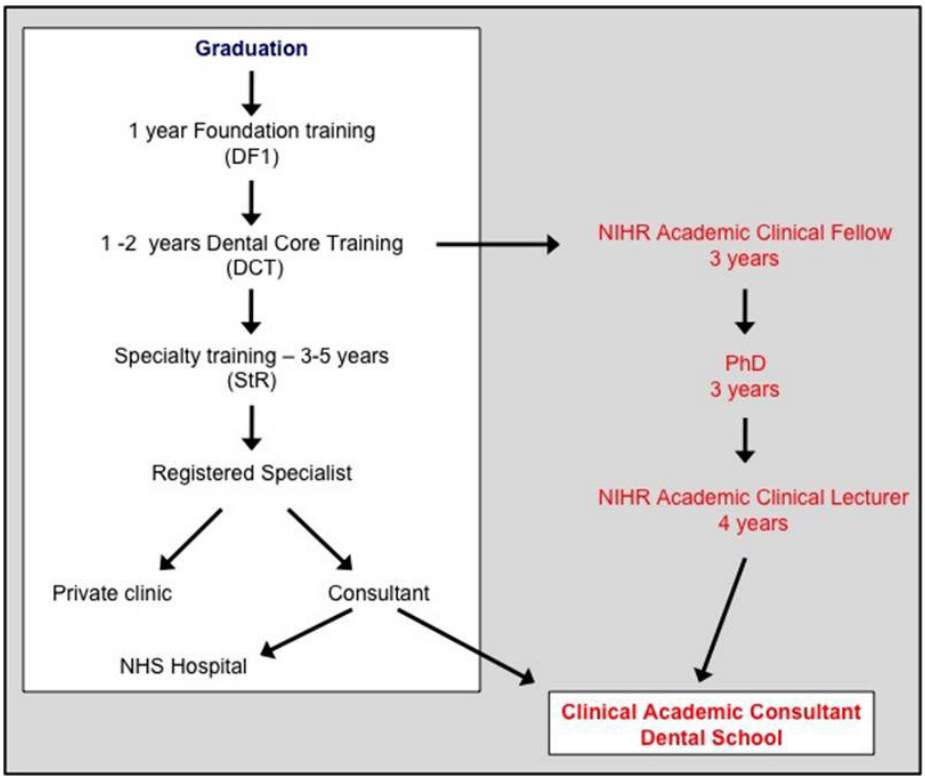

Figure 1. Postgraduate training pathways for dentists.
The career pathway in the white box is a purely clinical training within the NHS - ultimately leading to a consultant post in an NHS hospital. This takes upto 8 years from graduation. The pathway in red shows the Integrated Academic Training for progression to a senior academic post as a clinician scientist (see text for explanation). This takes up to 12 years.

They are a crucially important organisation because they bridge the gap between NHS clinical training and University academic training. As well as project grants and personal fellowship awards the NIHR also organise the *Integrated Academic Training* (IAT) programme. This provides funds so that dental clinical trainees can have time out from their clinical training to undertake research in the Dental Schools. These posts provide a pathway of training for clinician scientists (Figure 1) and are one of the main routes towards a clinical academic job in a UK University. There are two levels of post available. *Academic clinical fellows* (ACF) are junior pre-doctoral posts and provide funds for the clinical trainee to spend 25% of their time undertaking generic research training, usually toward a Master's in Clinical Research Degree (MClinRes). *Academic Clinical Lecturers* (ACLs) are post-doctoral positions and provide funds for the clinical trainee to complete their clinical training and spend 50% of their time undertaking post-doctoral research. At the end of an ACL post most dentists will have become eligible to apply for a senior academic post as a senior clinical lecturer.

Royal Colleges

There are three Royal Colleges of Surgeons (RCS) in the UK (England, Edinburgh and Glasgow), as well as a number of more specialist colleges including the Royal College of Pathologists. The RCS provide the specialty examinations that all trainees must sit and pass before they can be registered as a specialist with the GDC. In effect the GDC contracts the RCS to examine the trainees on their behalf. Most examinations are taken after 3 years of training and are called membership examinations and when passed the dentist becomes a Member of the Royal College in their specialty (eg. Membership in Orthodontics, MOrth). After 5 years of training the trainee sits the Fellowship examinations and becomes a Fellow of the royal College in their specialty. The Fellowship examinations are usually a requirement before being eligible for a NHS consultant post.

When the trainee has completed their training period AND passed the membership or fellowship examinations, the Dental Postgraduate Dean will certify that they have completed training and award a *Certificate of Completion of Specialist Training* (CCST). This is then approved by the GDC who will admit the dentist to the specialist register. The Dentist is then permitted to practice as a Specialist.

University Dental Schools

The Dental Schools train all undergraduate dentists and provide academic training for specialist trainees. However, unlike in many other countries including USA and Japan, the Dental Schools in the UK do not run a clinical service and do not have direct access to patients for teaching. Clinical teaching is carried out in an NHS hospital and is funded by HEE (see above). There are 18 Dental Schools in the UK and all are closely associated

with a teaching hospital. All clinical academic staff in the Schools also have a contract with the NHS, so that they can undertake clinical service and teach clinical subjects to undergraduate and postgraduate students. This means that, in their capacity as academic consultants, many Dental School clinical staff also train specialists. However the Universities cannot award qualifications that enable dentists to register as a specialist with the GDC: this can only be done by the Royal Colleges (see above).

The dental schools usually offer three types of postgraduate qualifications:

PhDs : These are conventional research training degrees similar to those in other parts of the world. They are available to clinical and non clinical candidates, and take 3-4 years to complete. It is very difficult to get funding to do a PhD and dentists have to apply for fellowships from the research councils or from NIHR. The Integrated Academic Training pathway (Figure 1) is funded by NIHR and provides support for dentists who wish to do a PhD.

Masters Degrees : Most dental schools offer 1 or 2 year masters programmes in specific clinical disciplines. These are often taken by dentists who wish to enhance their skills, but they do not lead to a specialist qualification which the GDC will recognise. However a masters training may contribute towards a specialist training, but the dentists will also have to satisfy the requirements of the Dental Postgraduate Dean and sit the examinations of the Royal Colleges. Many Schools collaborate with the NHS, the DPGD and the Royal Colleges to provide a unified training, which results in a Master's degree and eligibility to sit the College examinations. Some schools also offer MClinRes degrees, which provide generic research training for junior clinicians and ACFs.

Professional Doctorates : Some Schools offer a three year integrated clinical and academic training which results in a professional doctorate (Doctorate of Clinical Dentistry, DClinDent). These take from 3-4 years and are usually tailored to provide the clinical training required to satisfy the DPGD, and enable the candidate to sit the membership examinations of the Royal Colleges. Note however that the DClinDent itself is not recognised by the GDC and the dentists must ALSO sit the College examinations if he or she wishes to be registered as a specialist.

TRAINING PATHWAYS

Training pathways for dentists are summarised in Figure 1. It can be seen that it may take up to 12 years to become a senior clinical academic (clinician scientist) in a UK University. It is also a complex process involving multiple organisations and regulators as summarised above. Although salaries are reasonable (Specialty trainees may earn £30,000 - £40,000 GBP (~£50,000USD)) they are low compared to private practice and the training is long. This presents a formidable challenge to the aspiring specialist dentist and relatively few ever embark on this pathway. Less than 5% of dentists pursue an academic career making recruitment into Dental Schools very difficult.

On graduation, all UK dentists must complete a year of Foundation Training. This is carried out as an employee of the NHS in a dental practice. Foundation Dentists are taught by experienced dentists and learn management skills and become more proficient at whole patient care. The Foundation Programme is organised and assessed by Dental Postgraduate Deans, who work for HEE (see above).

On completion of foundation training the dentist is permitted to work for the NHS and most will then seek employment in primary care dental clinics. Only about 15% to 20% undertake further training, and overall, only about 10% will become a specialist. Those who wish to undertake further training will work in a hospital as a Dental Core Trainee (DCT) for one or two years. These posts are at a junior level and usually rotate through a number of clinical specialties.

Specialty training is undertaken in a Specialty training Registrar (StR) post in an NHS hospital. These are nationally competitive and are funded by the NHS through the Dental Postgraduate Deans. Training takes from 3-5 years (Table 1) and is supervised by a senior clinician (consultant) who is qualified to train. Training is continuously assessed. To complete the training and become a specialist a dentist must:

- be appointed to a recognised StR post
- complete the required amount of time in training (3-5 years)
- complete and pass about 18 workplace based assessments each year
- maintain a portfolio of all work done
- attend and satisfactorily pass an Annual Review of Competence Progression (ARCP)
- pass the relevant Royal College specialty examinations
- present the portfolio of work and pass the final ARCP for the DPGD to approve the award of a CCST (Certificate of Completion of Specialist Training)
- be placed on the GDC specialist register

Once registered, the specialist may be eligible to apply for a Consultant post in the NHS.

If the dentist wishes to work in a University and undertake teaching and research, they may seek a post which enables them to undertake research and study for a PhD. Some academic posts are funded by NIHR (ACFs and ACLs) and allow the dentist to do clinical training and research at the same time. These are shown in red in Table 1. To do a PhD the dentists must apply for funds to get a fellowship to allow them to take time out of clinical training for research. Organisations such as the NIHR, the Medical Research Council and a number of charities provide funds for fellowships. Dental Schools may also provide funding for academic training posts and for PhD study.

On completion of the clinical training and the PhD the dentist is then a eligible to apply for a post as an academic consultant at the grade of Senior Lecturer (Associate Professor). However, these posts are very competitive and you would require a research track record with high quality publications. Many newly qualified specialists therefore work in junior university posts

(lecturer) to build up their research and teaching experience.

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International Collaborative Program for Human Resources in Dental Medicine at Graduate-level: The Korean Perspective

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Abstract

Today, graduate education in dental school has become increasingly important for the advancement of dentistry. The goal of graduate education in dentistry is now focused on finding new knowledge and developing new technology for the advancement of human wellbeing in the field of dentistry. In Korea, 11 dental schools offer graduate programs. Large proportions of domestic students admitted to graduate programs are mainly from non-dental fields, such as biology or engineering. International collaboration needs more active engagement and mutual communication between multinational institutions. There are many issues in international collaboration. Interdisciplinary collaboration would be a very important preceding requirement of international collaboration. Dual degree programs, short-term exchange programs, and exchange seminars or conferences are possible and executable actions. Web-based open communication or on-line research seminars can be helpful for the expansion of collaboration. The most important goal of the graduate program is human resource development. Our goal of international collaboration should be to set balanced networks between involved nations. The institutional preparedness for international collaborations should be properly assessed in terms of expected outcomes and goals of individual schools. For this agenda we need a framework for human resource development, a consortium for the proper international collaboration and qualification of graduate education.

Introduction

Today, graduate education in dental school has become increasingly important for the advancement of dentistry. The goal of the graduate education in dentistry is now focused on finding new knowledge and developing new technology for the advancement of human wellbeing in the field of dentistry. In Korea, 11 dental schools offer graduate program including master of science (in Dentistry) and Ph. D. (in Dentistry) There are also departments of dentistry in many medical schools offering graduate degree programs in the clinical field. In graduate dental education, there are a large number of majors in both clinical dentistry and the basic science field. Degree granting graduate programs are run by the department of dentistry or dental science in the graduate school of each university.

In Korea, there are 10 specialties in clinical dentistry (specialty certificate). All of them also offers degree granting courses (MS and PhD) - Oral medicine and oral diagnosis, Oral and maxillofacial radiology, Oral and maxillofacial surgery, Pediatric dentistry, Orthodontics, Conservative dentistry, Prosthodontics, Periodontology, and Oral Pathology. In the basic science field, there are traditional majors - Anatomy, Physiology, Pharmacology, Microbiology, Biochemistry, Dental biomaterials, and Preventive dentistry. Some schools offer convergence majors, such as Head & Neck Anatomy and Imaging Science, Immunology and Molecular Microbiology in Dentistry, Molecular genetics, Cell and Developmental biology, Neuroscience, Preventive and Social dentistry, Healthcare management and informatics, Cancer and developmental biology, Dental biomaterials science and Dental regenerative biotechnology. Each master and doctorate degree program require a minimum 2 years course. Some master-doctorate combined programs need a minimum of 4 years. But the total course length is determined by the research progress of each theme.

The graduate student selects an advisor professor in the department within 1 year after the student enters the school. After completion of the required courses, the student must pass the qualifying exam before submission of thesis. For international students, they must pass a Korean or a second language exam (except their mother language). The student will be eligible to submit his/her thesis, only if he/she publishes a paper in the international journal recognized by our graduate school. During the course, the student will engage in a research project with their advisor. There will be plenty of chance to participate in intramural research seminars and national and international meetings.

The challenges and perspectives of education at graduate schools

Graduate-level training in Korean dental school previously was mainly focused on developing practice skills. There were also graduate programs for basic science research, but they were not enough. Many graduates were looking to other countries to get advanced graduate education.

With the economic growth of the nation, the increase in national economic power has brought an improvement in research capacity. In recent years, dentistry has become one of the most rapidly advancing field of profession in Korea. Dentists promptly adopted lots of newly developed technology and research results quickly. With the economic growth, the opportunities for

postgraduate education has increased gradually. The focus of graduate programs in academic institutions also shifted into the field of science and technology. Many Korean dental education institutions are now trying very hard to become a research-focused academic institution. Large proportions of domestic students admitted to graduate programs are mainly from non-dental fields, such as biology or engineering.

Graduate education aims to develop dentistry through research and education for dentistry, natural sciences and engineering and serve the nation and mankind by training talented experts at an international level.

Korean dental schools aim to develop dentistry through academic research in which the dentistry is combined with students who have various and broad academic backgrounds. We aim to gain international competitiveness in each area by creating a healthcare environment in which research, education and treatment are efficiently coordinated. We are making efforts to improve the welfare of mankind by performing research and education with the responsibility to develop dentistry once we have recognized the implementation of education philosophy as national project and our responsibility. For these goals, we need an expansion in international collaboration. But until now these efforts have only gained limited success.

Issues in international collaboration

Korean dental education institutions traditionally sent many graduates to developed countries in both clinical and basic science research areas. Majority was postgraduate specialty training, but also some students went abroad in research fields.

Incoming student population has grown rapidly in recent years. But our system of opportunity is still limited. We have government-run national scholarship programs. But it has very limited numbers. University-based scholarship programs have also increased significantly. But both programs are only eligible to limited countries. This made studying in Korea unattractive. Now, we are developing school-based scholarships and research grant conjunct scholarship programs. But international collaboration of graduate education cannot be completed only by scholarship programs. International collaboration needs more active engagement and mutual communication between multinational institutions. Though we believe that international collaboration leads to better dental science, our institutional budget and funding structures are 'always' not enough to support international collaboration. The students need more opportunity to meet each other. How can it be accomplished?

There are other important issues in international collaboration within graduate education. For graduate education and international collaboration, the clinical field always got caught in license issues. Language is also a big barrier. Cultural issues need to be considered as well. There is also the issue of age and gender. Terms of visit will also be an important issue. We need to evaluate prospective outcome of short-term (weeks or months) or long-term (full-course or years) programs. Sometimes the differences between countries are less serious than departmental barriers. Interdisciplinary collaboration would be a very important preceding requirement of international collaboration.

Each country has a different priority in research topics. In developed countries the diversity of topics may not be a problem but developing or underdeveloped countries see it as an important issue. The meaning of research in each countries with different cultural and socioeconomic situation will be different. Academic program may be similar but also very different. Do we need standardization of the curriculum? Or can we grant diversity to each education program?

Followings are part of action plans for globalization of Seoul National University, School of Dentistry.

- To participate in the exchange of human resources (students and faculty)
- To expand a network of dental education institutions.
- To acquire advanced technologies and develop educational programs
- To play an active role in advancing dentistry in developing countries through fund-raising
- To host global academic conferences and symposiums
- To share knowledge and skills of dentistry globally
- To promote sustainable and diversified joint research

But the proclamation is not enough to reach the goal. We should consider detailed issues and make feasible action plans. Dual degree programs, short-term exchange programs, and exchange seminars or conferences are possible and executable actions. Sometimes web-based open communication or on-line research seminars can be helpful for the expansion of collaboration.

Suggestion

Globalization can be defined as 'the process by which markets and production in different countries are becoming increasingly interdependent due to the dynamics of trade in goods and services and flows of capital and technology.' (European Commission, 1997) The most important goal of the graduate program is human resource development. In any country, qualified human resources are the most important assets for the nation and the whole world. Internationalization always has a flow — unidirectional or bidirectional. But our goal of international collaboration should be to set balanced networks between involved nations.

The institutional preparedness for international collaborations should be properly assessed in terms of expected outcomes and goals of individual schools. For this agenda we need a framework for a human resource development.

I suggest a consortium for the proper international collaboration and qualification of graduate education. It can be an international supervisory committee which, works to evaluate each program for its preparedness to mutual understanding and investigates the impact of international collaborations.

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International Collaborative Graduate Program at the Faculty of Dentistry, Chulalongkorn University

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ABSTRACT

International collaborative management, with the help of professors from University of Alabama for the undergraduate program, is an integral part of the Faculty of Dentistry, Chulalongkorn University since its inception in 1940. The first graduate program was established in 1973 with the support from the University of Alabama and there have been many graduate programs established due to the collaborative management with the school overseas. In 2008, a Master's Degree in Esthetic Restorative and Implant Dentistry (International Program) was started in collaboration with the School of Dentistry, UCLA. The program integrated advance art and science into several major fields in dentistry to improve or maintain esthetic condition of the patient. Starting in 2016, a Doctor of Philosophy in Dental Science (Orthodontics) will be started as a result of collaboration between Chulalongkorn University and Tokyo Medical and Dental University and it will be CU-TMDU Joint Degree Program. With strong collaboration between universities and the usage modern information technology, this will enable the development of dental education in terms of inter-disciplinary education with long-term international collaborative management.

INTRODUCTION

The genesis of the dental curriculum in Thailand was initiated in 1928 by Professor Colonel Luang Vach Vidyavaddhana, with the objective of providing dental education and establishing a school of Dentistry in Thailand. After he received his medical degree from Syracuse University he furthered his study in Dentistry at the University of Pennsylvania. When he returned to Thailand, he asked Professor Aller Gustin Ellis, the president of Chulalongkorn University to set up the school of Dentistry, however due to shortage of budget it was unsuccessful. He then proposed to the head of the Ministry of Defense, Field Marshal P. Pibulsongkram, with the idea of setting up the school of Dentistry to provide services for military personals. He then received a travel grant from the ministry of defense for academic tour overseas in order to learn more about what was needed to set up a school of Dentistry. After coming back from the academic tour Field Marshal P. Pibulsongkram has became the president of Chulalongkorn, and as a result the project for the army was shifted to a development of a school of Dentistry within Chulalongkorn University instead and in 1940 Professor Colonel Luang Vach Vidyavaddhana was appointed to be the first head of the new section. In 1943, the University of Medical Sciences was founded and the dental section in Chulalongkorn University was included so the affiliation of the first dental school was changed. Professor Colonel Luang Vach Vidyavaddhana then became the first dean of the Faculty of Dentistry in the University of Medical Sciences. In 1969 the University of Medical Sciences was renamed into Mahidol University and in 1972 the school of Dentistry returned to Chulalongkorn University where it has located until this day.

At the beginning there was only three faculty staff, Professor Colonel Luang Vach Vidyavaddhana decided to write a letter to the US government for academic support. Professor Joseph Volker, the dean of the School of Dentistry, University of Alabama, answered his request and was sent by the US government to Thailand to help train the faculty staff and teach the students. At that time, curriculum development was also conducted. It cannot be claimed that the first international collaborative program was initiated, it was however the first dental collaboration between Thailand and the United State for the undergraduate program. Many faculty staffs from Chulalongkorn University were sent to study in the University of Alabama and later on Professor Charles Macallum also came to help the Faculty of Dentistry, Chulalongkorn University. As an oral maxillofacial surgeon he provided knowledge and collaborations, which leads to the establishment of specialty training in Maxillofacial in 1973 by Chulalongkorn staffs, which were inspired by Professor Charles Macallum and Professor Chuachote Hungsasut was trained at the University of Alabama and finally become the first Thai dentist who got the first Diplomate, American Board in Oral and Maxillofacial surgery. With Professor Macallum's enormous support, he was honored with an honorable degree of Doctor of Philosophy from Chulalongkorn University in 1993. It is the first graduate program in Thailand which was established from the collaboration between Chulalongkorn University and the University of Alabama.

Many more graduate programs ranging from 1 year up to 5 years were introduced and more than 20 graduate programs are currently available. In 2008, the first international collaborative master degree program was started. It is a Master's Degree in Esthetic Restorative and Implant Dentistry (International Program). The objectives of this program are to

1. Establish the international teaching and learning courses.
2. Enable the faculty to generate a program that is self-supported.
3. Establish a national standard for international dental education.
4. Establish a prototype for dental service that is different from the governmental pattern.

The esthetic restorative and implant dentistry is the advanced art and science of integration of several major fields in dentistry, i.e. operative dentistry, prosthodontics, periodontics, surgery, orthodontics and occlusion, involving systematic treatment planning to achieve highly successful treatment outcome leading to proper function of temporo-muscular system, as well as improving or maintaining esthetic condition of the patient. The program provides services, both by students and faculty members, to patients that would promote not only their oral and physical but also mental health. Collaboration with universities abroad such as University of California at Los Angeles was established. Students attending the program will spend at least one semester at sister dental school which is at present the students will visit School of Dentistry, UCLA to get extra training for one semester. The students will be able to attend lectures, seminars and also clinical observation. They will sit in the class with those students in UCLA.

Curriculum Structure composes of 42 credits with required subject of 18, electives of 2, clinical and laboratory practices of 10 and 12 for Thesis. For the non-thesis program it composes of required subject of 24, electives of 2, clinical and laboratory practices of 10 and 6 for independent study (clinical-oriented). The duration of study is 2 academic years which include 2 summer sessions. The program also produces a number of publications.

For the thesis program, the study plan is as follow:

Year 1 Semester 1 (12 credits)
- Research Methodology in Dentistry
- Statistics in Dentistry
- Conservative Dentistry Art
- Appreciation for General Esthetics
- Principle of Esthetic Dental Treatment I
- Principle of Implantology I
- Seminar in Esthetic Dentistry I
- Seminar in Implantology I
- Implant Dental Laboratory
- Esthetic Dental Laboratory

Year 1 Semester 2 (11 credits)
- Principle of Esthetic Dental Treatment II
- Diagnosis and Treatment Planning for Esthetic Restorative Dentistry and Implantology
- Dental Material Science for Esthetic Dentistry andImplantology
- Seminar in Esthetic Dentistry II
- Esthetic and Implantology Dental Clinic I
- Seminar in Implantology II
- Thesis
- Elective

Year 2 Semester 1 (9 credits)
- Principle of Implantology II
- Seminar in Esthetic Dentistry III
- Esthetic and Implantology Dental Clinic II
- Seminar in Implantology III
- Thesis
- Elective

Year 2 Semester 2 (10 credits)
- Esthetic and Implantology Dental Clinic III
- Thesis

For non-thesis program, study plan is as follow:

Year 1 Semester 1 (10 credits)
- Research Methodology in Dentistry
- Statistics in Dentistry
- Conservative Dentistry
- Art Appreciation for General Esthetics
- Principle of Esthetic Dental Treatment I
- Seminar in Esthetic Dentistry I
- Esthetic Dental Laboratory
- Principle of Implantology I

Year 1 Semester 2 (11 credits)
- Seminar in Implantology I
- Implant Dental Laboratory
- Principle of Esthetic Dental Treatment II
- Dental Occlusion: Principle and Application for Restorative Dentistry
- Diagnosis and Treatment Planning for Esthetic Restorative Dentistry and Implantology
- Dental Material Science for Esthetic Dentistry and Implantology
- Seminar in Esthetic Dentistry II
- Esthetic and Implantology Dental Clinic I
- Seminar in Implantology II
- Elective

Year 2 Semester 1 (11 credits)
- Principle of Implantology II
- Seminar in Esthetic Dentistry III
- Seminar in Implantology III
- Esthetic and Implantology Dental Clinic II
- Multidisciplinary Approach to Advanced Esthetic Dentistry
- Independent Study in Esthetic Restorative and Implant Dentistry I
- Elective

Year 2 Semester 2 (10 credits)
- Esthetic and Implantology Dental Clinic III
- Independent Study in Esthetic Restorative and Implant Dentistry II
- Technology in Esthetic Dentistry
- Multidisciplinary Approach to Advanced Implantology

The program has been continued for 6 classes starting from academic year 2008 until at present (stop for curriculum development for one year). There are 41 students graduated from this program and another 11 students are studying in the program now.

In 2013, another idea was initiated for another international collaborative graduate program. It is a PhD program with collaboration between Chulalongkorn University and Tokyo Medical and Dental University. The program is now under development by both universities, affirming its long history of collaboration. It is the Doctor of Philosophy in Dental Science (Orthodontics), CU-TMDU Joint Degree Program. Students can enroll

from both sides but follow the same process. The program was designed to bring strengths of the two universities and share those strengths for students. The students will enroll in course work, practical exercise and dissertation seminar in Thailand for 1 year while they will do qualifying examination and also dissertation seminar for the second year. The students will study using teleconference with Japan through the third and fifth year so they can concentrate on their course work and clinical training in Thailand, which include proposal defense, dissertation seminar and dissertation examination. However, the final agreement for all details has not been finished yet. The 5 Year Program (72 credits) will comprise of:

Course work 24 credits	
Lecture	8 credits
Practical exercise	1 credit
Seminar	2 credits
Clinical training	12 credits
Elective	1 credit
Dissertation 48 credits	

This will open a new era of collaborative dental education, with ample usage of information technology while keeping rooted in traditional study and clinical trainings. It will expand expertise and share resources to bring out the most efficient way of teaching and research on the international level. The program is expected to be completed and will start at the earliest in the academic year 2016. There are many international graduate programs at the Faculty of Dentistry, Chulalongkorn University in which there is no collaboration with other local or overseas dental institutions. The two programs are academic innovation that differs from other specialty programs from other dental schools.

CONCLUSION

In conclusion, with strong collaboration between universities and modern information technology, it is possible to conduct an international collaborative graduate program to strengthen knowledge sharing and research collaboration. This will enable the development of dental education in terms of inter-disciplinary education with long-term international collaborative management.

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Session IX

Oral Health Promotion and Functional Rehabilitation in Super-aged Society

Initiatives toward the Prevention of Long-Term Care Dependency in Japan

Ministry of Health, Labour and Welfare
M. Sakoi

Oral Rehabilitation and Regional Partnerships

Nippon Dental University
F. Tamura and T. Kikutani

What Can the Dentistry Do in Super-aged Society?

Hiroshima University
M. Yoshikawa

The Role of Dental Professionals in Dysphagia Rehabilitation

Tokushima University
M. Matsuyama

Initiatives toward the Prevention of Long-Term Care Dependency in Japan

M. Sakoi

M.D., M.P.H., Director, Aging and Health Division, Health and Welfare Bureau for the Elderly, Ministry of Health, Labour and Welfare, Government of JAPAN

Changes in Japan's Population

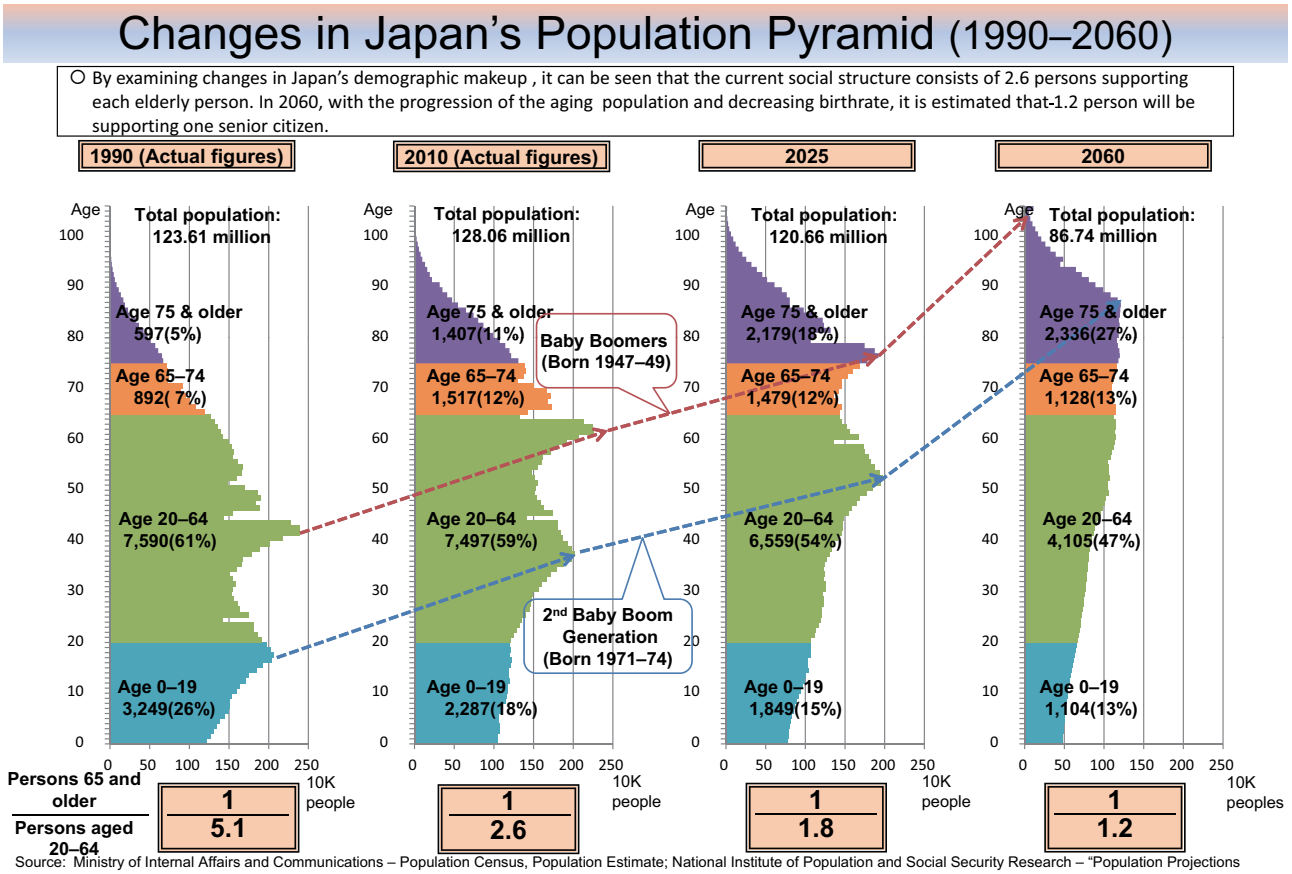
By examining changes in Japan's demographic makeup, the number of seniors over age 65 is predicted to reach 36.57 million by 2025 and reach a peak of 38.78 million in 2042. Additionally, the percentage of seniors over age 75 is expected to grow, surpassing 25% by 2055. It can be seen that the current social structure consists of 2.6 persons supporting each elderly person. In 2060, with the progression of the aging population and decreasing birthrate, it is estimated that 1.2 person will be supporting one senior citizen. (Fig. 1)

Since the establishment of the long-term care insurance system in 2000, the population over age 75, as an age group with high percentage of persons requiring care, has increased rapidly and the rapid increase will continue for the next 10 years to 2025. From about 2030, the rapid growth of the population over age 75 will level off but the population over age 85 will continue to increase for another 10 years. The population over age 40, who pay for the long-term care insurance, has increased since the establishment of the long-term insurance system in 2000 but will start to decrease after 2025.

Among seniors over age 65, those over level II of the "daily life independency level for seniors with dementia" will increase. Individual or couple-only households with householders over age 65 will increase. The number of seniors over age 75 will rapidly grow in cities and gradually grow in rural areas with originally high senior population. Tailored response according to regions is necessary as aging circumstances differ according to region.

Establishing the Integrated Community Care System

By 2025 when the baby boomers will become age 75 and above, we will implement the creation of a structure (comprehensive regional care system) that comprehensively ensures the provision of health care, nursing care,



Source: Ministry of Internal Affairs and Communications – Population Census, Population Estimate; National Institute of Population and Social Security Research – "Population Projections for Japan (January 2012): Medium-Fertility & Medium-Mortality Assumption" (Figures as of Oct. 1 of each year)

Fig. 1

prevention, housing, and livelihood support so that the elderly could live the remainder of their lives in their own ways in environments familiar to them, even if they become heavily in need for long term care. (Fig. 2)

As the number of seniors with dementia is expected to rise, the creation of the Integrated Community Care System is important to support the local living of the elderly with dementia. The progression status will have regional differences; large cities with stable total population and rapidly growing population of over 75, and towns and villages with gradual increase of population over 75 but decrease of total population. It is necessary for the municipalities and prefectures that serve as insurers to create the Integrated Community Care System according to the regional autonomy and independence.

Enhancement of Livelihood Support, Social Participation of the Elderly

With the increase of single households and seniors with mild need of assistance, the necessity for livelihood support is expanding. It is needed for diverse entities such as volunteer groups, NPOs, private corporations, and cooperative associations to provide livelihood support services. Preventive long-term care for seniors is needed. Social participation and social roles lead to long-term care prevention, and purpose of life. Systematic positioning regarding municipalities supporting the creation of communities providing various livelihood support, should be strengthened. Specifically, the development of regional resources such as the training and discovering of livelihood supporters (e.g. volunteers), and the allocation of "livelihood support coordinators" who do the networking, should be positioned under the Community Support Project of the Long-Term Care Insurance Act. (Fig. 3)

Aims of Long-Term Care Prevention and its Future Directions

Long-term care prevention aims to provide each senior with lifelong self-actualization and a purpose in life (i.e., improvement of QOL) by improving health condition and facilitating participation in social activities via environment adjustments and improvement of body functions. There is an apparent trend showing that the higher the percentage of social participation (such as sports-related groups, volunteering, and hobby-related groups), the lower the risk of falls, dementia, and depression.

Not only are the direct approaches to the elderly themselves, but also the well-balanced approaches that include the environment surrounding them are important. Approaches contributing to self-reliance that utilize rehabilitation professionals in the community and the community development by developing community activities run by the community members themselves have to be promoted. Similarly, a new social role in the community which will consequently link to the prevention of long-term care, and lead to a synergistic effect, needs be given to the elderly. It is essential for the actual conditions of the communities to be understood, and for

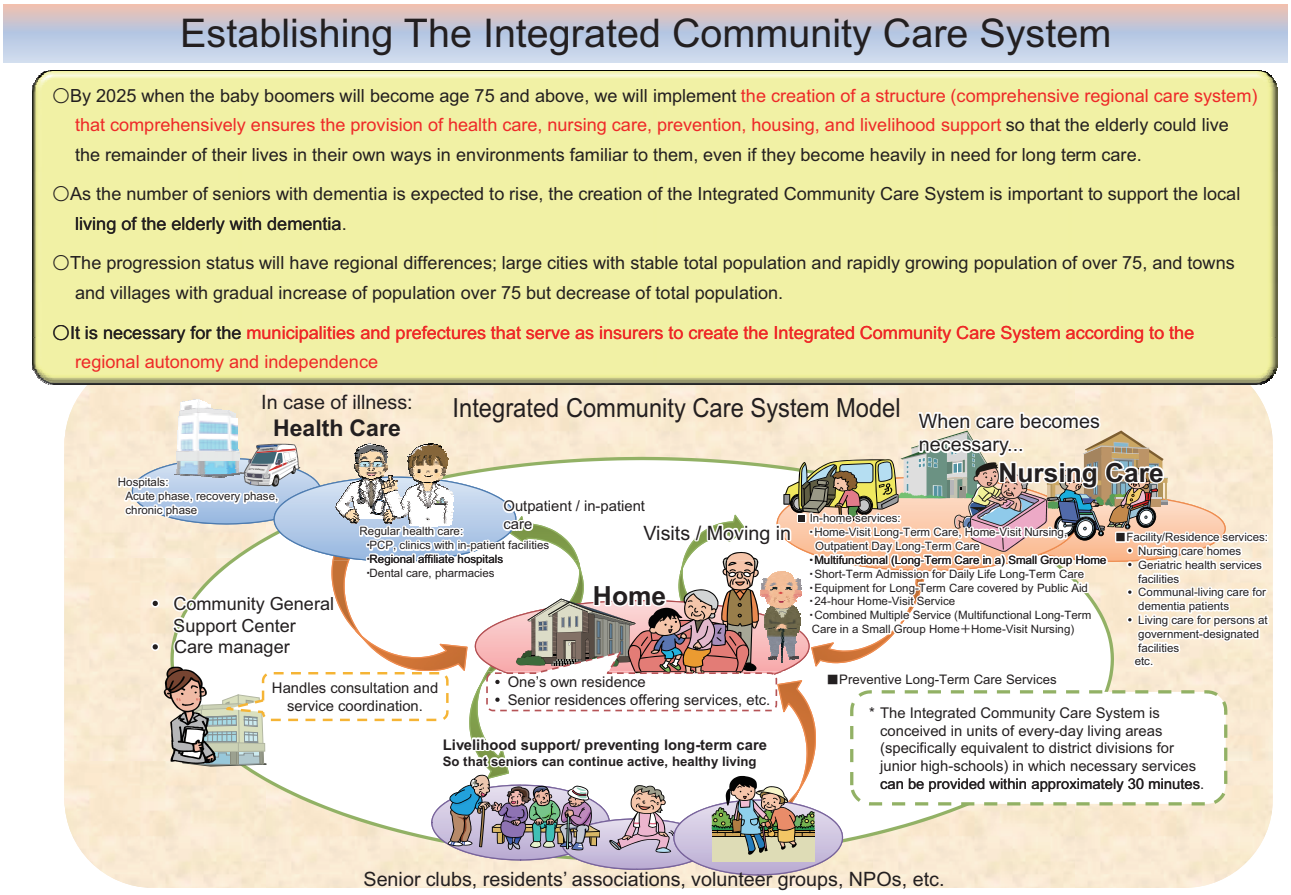


Fig. 2

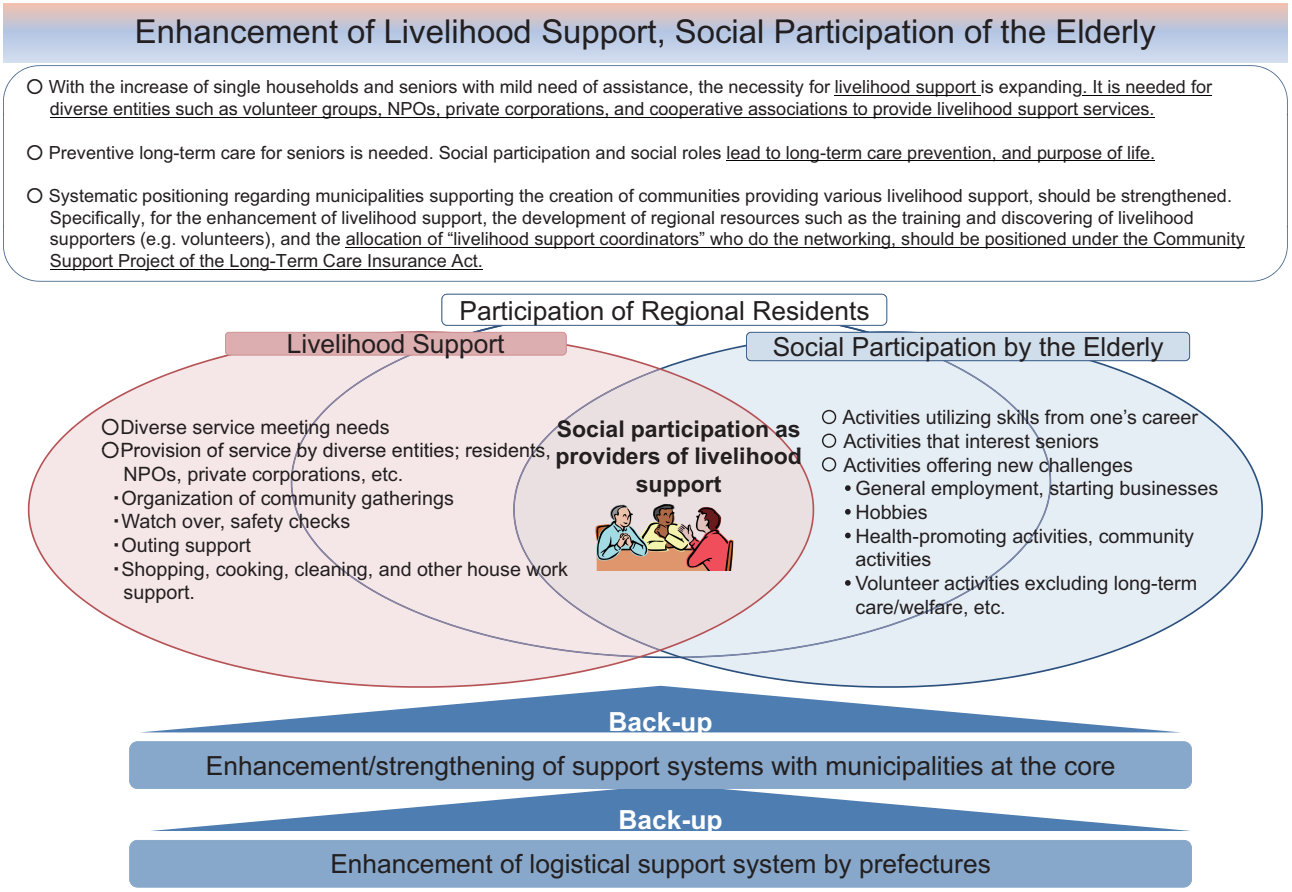


Fig. 3

the municipalities that work as the center of community development to take action proactively.

With respect to specific future approaches for long-term care prevention, it is important to enhance functions of long term care dependency prevention by utilizing rehab professionals and resident-operated commuting, especially through social participation by seniors.

Oral Rehabilitation and Regional Partnerships

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ABSTRACT

Japan is becoming a super-aged society, with many elderly people dying while hospitalized. However, not a few elderly people want to spend the later stages of their lives at home. In at-home settings, various types of medical and dental services, as well as visiting nurse's stations and pharmacies, etc., are required to support the elderly. In Japan, dentists and dental hygienists are typically involved in the treatment of adult and pediatric patients with dysphagia. Furthermore, most bedridden elderly people and physically disabled children reportedly suffer from dysphagia. Our clinic, which specializes in the treatment of dysphagia, was established in 2012, and now annually serves 3,000 patients. By employing a simple and convenient training device, the Pecopanda®, together with a tongue pressure measurement device, we have experienced successful oral functional training cases. In practicing nursing care, however, the provision of care should be interlinked among services through multidisciplinary cooperation. Construction of a comprehensive dysphagia treatment system is expected for the support of people living in the community.

INTRODUCTION

Japan is now becoming a super-aged society. The so-called "baby boomers" have become the older generation. Furthermore, in 2015, the number of demented elderly is estimated to be 2.5 million. Single-person households will account for 33% of all households defined as aged. The estimated annual death toll is anticipated to exceed 1.4 million, reaching a peak of 1.7 million (by Japanese Ministry of Health, Labour and Welfare). The percentage of people dying at medical institutions is also increasing annually, and, in 1976, exceeded the percentage of those dying at home, having reached a level of more than 80% in recent years (by Japanese Ministry of Health, Labour and Welfare). In other words, most elderly people nowadays die while hospitalized. Under these circumstances, the issue of where people should be attended at the time of death is anticipated to become a major problem in the near future. On the other hand, many people want to spend the later stages of their lives not in a hospital, but at home and even in their own home towns.

Medical and Dental Approaches for Community-dwelling People in Japan

In order to provide support care for patients, a hospital has a nurse's station, a pharmacy, a patient support department and so on, in addition to examination rooms for internal medicine, surgery, otorhinolaryngology, ophthalmology, dentistry, etc. In at-home settings, on the other hand, a town can be compared to a hospital, and a street of the town, to a hospital corridor. Located along the street are an internal medicine clinic, a surgical hospital, an otorhinolaryngological clinic, a dental clinic, a visiting nurse's station, a pharmacy, a nursing care support service office, and so on, all operating to support care needs and recuperation at-home. The number of institutions providing home-visit dental services at nursing care facilities has gradually increased. In contrast, the number of institutions providing home-visit dental services at the homes of patients has decreased. This indicates that, of the visiting care services currently provided, care at home has clearly fallen behind.

Most of the bedridden elderly reportedly have problems with eating. How many patients with dysphagia need help in Japan these days? The surveys conducted by the Japanese Ministry of Health, Labour and Welfare in 2012 demonstrated that 1,235,000 people had suffered a stroke and 512,000 had a diagnosis of dementia. Most were at risk for concurrent dysphagia and aspiration pneumonia. In fact, pneumonia is presently the third leading cause of death. On the other hand, among children, 1,810,000 physically disabled children were living at home in 2012. Those with severe motor and intellectual disabilities, suffering multiple physical and mental disorders, often have dysphagia.

As a unique feature of Japanese dental practice, dentists and dental hygienists are involved in the treatment of patients with dysphagia as members of a multidisciplinary team. This is specific to Japan, and differs especially from the practices prevalent in Europe and the United States.

The Japanese Society of Dysphagia Rehabilitation was established in 1995. Currently, the society is comprised of over 12,750 members. The most common occupation for members of this society is the speech pathologist: 4,150 members (32.5 %), second is the dentist: 2,269 members (17.8 %), and next is the dental hygienist: 957 members (7.5 %). Thus, many Japanese dental practitioners are involved in the treatment of patients with dysphagia, with numbers similar to those of speech pathologists in the United States.

Our Approach to Oral Rehabilitation

Next, we introduce our approach to the fields com-

prising oral rehabilitation. We established the "Tama Oral Rehabilitation Clinic of The Nippon Dental University" on the 16th of October 2012, with the aims of "giving at least one spoonful of food to care receivers" and "letting disabled children eat food just as well as other children". These are the dreams to which we aspired. In response to the requests of Department leaders concerning our vision for the future, Professor Kikutani, the director of this clinic, proposed the establishment of a center specialized in swallowing disorder treatment, because there had previously been no such a center in Japan. The proposal was accepted by the Dean, and one year later, our new clinic was established.

Our clinic currently serves 3,000 new adult and pediatric patients with dysphagia. Stroke (28%) and dementia (16%) account for the majority of the causative diseases. As for treatment styles, outpatient and visiting treatments account for 42 and 58%, respectively, including both those at facilities and those provided at home.

Many methods are available for oral-function and dysphagia rehabilitation, and the most suitable rehabilitation style is selected based on the patient's symptoms and receptiveness. Among these options, the Pecopanda® (JMS Co., Ltd., Tokyo, Japan), which is a simple and convenient training device for improving tongue pressure, is effective if the patient is capable of taking an active approach to achieving improvement[1]. A medical device for tongue pressure measurement has also been developed[2-4]. The following is a case that we experienced in the region around our clinic, in which a remarkable effect was achieved in response to oral functional training using these instruments and measurement devices.

Case: Preparation of Dental Prosthesis and Oral Rehabilitation in a Patient with Swallowing Disorder due to Crossed Hemiplegia after Brain-stem Hemorrhage

Crossed hemiplegia is defined as unilateral hemiplegia accompanied by contralateral cranial nerve palsy after brain-stem impairment. We experienced a patient who had oral motor dysfunction[1,5] and his eating activities were improved by preparation of a dental prosthesis and oral rehabilitation. This patient had oral sensory paralysis on the right side and motor paralysis on the left side, in a crossed manner after brain-stem hemorrhage, resulting in oral function disorder and inability to use a dental prosthesis.

The patient was a 66-year-old man with edentulous jaws. Since suffering a brain-stem hemorrhage in 2010, he had not used a dental prosthesis prior to our evaluation of him in 2013. The edentulous state had necessitated ingestion of a paste diet. At the time of our first visit at his home, a simple evaluation of eating/swallowing functions was performed. Furthermore, in order to prevent food remaining on the side with the sensory deficit (Figure 1), dental prostheses for the upper and lower jaws with relatively thick prosthetic bottoms were prepared. After the dental prostheses had been completed, eating/swallowing functions were examined, and lingual resistance training using the Pecopanda® was performed with reference to the lingual pressure measured using a lingual pressure meter (JMS Co., Ltd.). To treat the

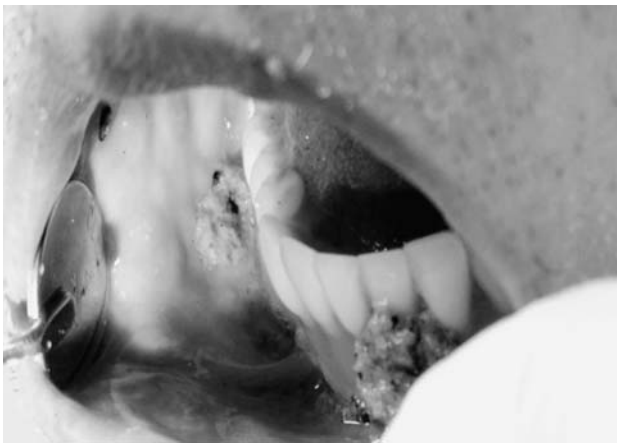

Figure 1. Food residue on oral vestibule of paralyzed side

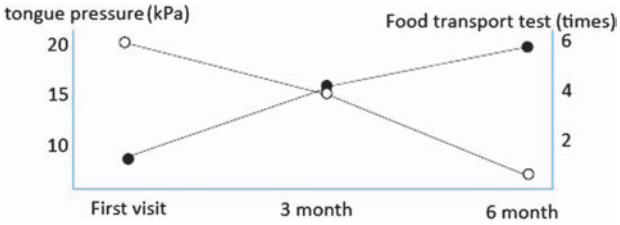

Figure 2. Improvement of lingual functions[1]

decrease in skilled lingual activities, intraoral transfer training using test foods was performed. In accordance with functional changes achieved by training, the dental prosthesis was continuously adjusted. Lingual pressure thereby increased and skilled activities of the tongue and lips showed improvement, enabling the patient to ingest a regular diet (Figure 2). This case suggests that the use of a dental prosthesis suitable for ameliorating oral dysfunction and achieving ongoing rehabilitation can contribute to improving quality of life for patients.

As mentioned above, the Pecopanda® device (easy to handle during the process of training, and the effect can be confirmed) and the tongue pressure measurement device (easily portable) were also found to be useful for providing home-visit dental services. However, the utility of these instruments is not limited to the elderly alone. For instance, in 2014, we asked an 8-year-old boy with Down syndrome, who was attending our clinic as an out-patient, to engage in tongue pressure training independently using the Pecopanda® device at home. We continuously measured his tongue pressure at our out-patient clinic, and succeeded in improving his swallowing function by motivating him. This motivation was achieved by providing the boy with opportunities to confirm the rehabilitation effect by himself.

CONCLUSION

What does it mean "to support people with eating at the place where they are living"? In our view, "eating" is a source of power in the field of nursing care. First and foremost, the circumstances in which people live influence the purpose and outcome of their oral rehabilitation. Another issue is that members of the multidisciplinary team belong to different fields, which can make mutual

cooperation difficult. One of the approaches to solving these problems might be the establishment of a comprehensive dysphagia treatment system in the community where people are actually living. In practicing nursing care, there should be interlinkage of all forms of care provided, extending from the hospital to the home. Attention should be paid not to "what people can do (are able to eat)", but to "how people are managing (are coping adequately with eating/dietary needs)". We believe that narrative-based nursing care is superior to evidence-based nursing care. This viewpoint may provide the foundation for a positive oral rehabilitation concept in the super-aged community.

ACKNOWLEDGEMENT

The part of this study was supported by Japan Agency for Medical Research and development, AMED. We thank Dr. Hiroki Shindo for his fruitful collaboration.

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What Can the Dentistry Do in Super-aged Society?

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Key words: super-aged society, gerodontology, swallowing disorders, oral rehabilitation

ABSTRACT

The aim of gerodontology is a "Recovery of eating function for the eldery" ultimately. And this is possible to restate "the dentistry for protection, maintaining and improvement of the oral function in the elderly as to be able to do oral intake until their last days". Aging of society is a universal problem[1], and Japan is at the forefront of fighting problems in super-aged society. Dysphagia caused by aging itself and general diseases issue definitely, when you pursue the Gerodontology. Dentistry can make a contribution to it through the oral rehabilitation[2].

Enhanced support to the burgeoning dementia population is also urgent issue in the world. It is predicted that over 50% of them have dysphagia[3], and immediate response to their dysphagia problems will be needed.

The more number of the elderly with care needs increase, the more number of the elderly who can't intake orally with deteriorated swallowing function increase. In addition, the number of aspiration pneumonia patients will definitely increase because of their poor oral intake.

I'd like to introduce the current reality in Japanese super-aged society and what we have to prepare for it in the near future.

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The Role of Dental Professionals in Dysphagia Rehabilitation

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Key words: Dental professional, dental hygienist, role, education, dysphagia rehabilitation, Super-aged Society

ABSTRACT

Japan has become a "super-aged" society. In 2014, the number of elderly people was 33 million, or 26.0% of the population. The number of dependent elderly was over 5.4 million, 20% of whom had difficulty swallowing. So to say, dysphagia is a common disease state of the elderly in Japan. Dysphagia can cause choking accidents and aspiration pneumonia. More than 4000 choking accidents happen every year and pneumonia is the largest cause of death among the elderly in Japan. Dysphagia rehabilitation is therefore becoming more widely used in nursing care to prevent aspiration pneumonia and choking accidents.

Dysphagia rehabilitation is performed by a team, including a doctor, dentist, nurse, speech therapist, dental hygienist, dietitian, radiation technologist, and pharmacologist. Dental hygienists are among the oral healthcare specialists needed before and during rehabilitation, and have a key role in oral functional rehabilitation. Dental professionals, including dental hygienists, are now expected to take a lead in oral healthcare, and therefore in dysphasia rehabilitation. The involvement of dental professionals in rehabilitation helps other medical professionals to improve and support eating function for the elderly.

BACKGROUND

Situation in Japan

Remaining healthy is important throughout life, which means that health promotion can and should be targeted at all age groups. Various oral health promotion measures could be used to target different age groups. These measures also need to consider the background, current situation, and general prognosis on a national level. The main target of oral health promotion can vary in different countries, often driven by the demographic situation. For example, in most ASEAN countries, efforts are likely to be focused on children and young people, because these countries tend to have an increasing birth rate and higher ratios of children to the elderly. In Indonesia, the percentage of the population aged 65 and over was 5.0% in 2012, the same as in Japan 65 years earlier[1] (see Figure 1). However, Japan is now a so-called "super-aged" society; its declining birth rate and aging population mean that the focus of oral health promotion tends to be on adults and the elderly.

According to the 2015 Annual Report on the Aging Society, the number of people aged 65 and over in Japan is now 33.0 million and the percentage of the population aged 65 and over has risen to 26.0%[1]. The number of the elderly with dementia has increased by 4.62 million, and the number with mild cognitive impairment was almost 4 million in 2012[2]. The number of people aged 65 and over who are certified as requiring long-term care was 5.457 million in 2012[3] (see Figure 2), and of these, almost 20% had swallowing problems (dysphagia)[4].

There were a number of measures of health, nursing care and medical care for the aging society in FY 2014[5]. These included promotion of nursing care prevention, medical care system for the elderly, integrated and sustainable provision of home medical and long-term care in the community and promotion of livelihood support through mutual help in regional communities.

The percentage of people aged 80 and over with at least 20 teeth reached 38.3% in 2011, largely because of the "8020" oral health campaign. We expect that the number of older adults with natural teeth and cognitive impairment is likely to increase, requiring more oral hygiene support from dental professionals.

Older adults' oral health status and condition could affect their quality of life[6]. Older adults recognize that having teeth or dentures could influence several aspects of quality of life, including their ability to eat a meal, chewing and their appearance[7], and that good oral health is important to eating and comfort[8]. For many older adults, having a meal is one of the most pleasurable events in their lives. Katoh et al[9] studied elderly people using various facilities including special nursing homes, healthcare services, geriatric hospitals and long term hospitals, and found that meals were considered more enjoyable than family visits or special events of various kinds. Eating meals can therefore be considered to make elderly people happy and improve their quality of life. However, eating is more dangerous than might be expected for the elderly with dysphagia.

Dysphagia can cause choking accidents and aspiration pneumonia. More than 4000 choking accidents happen every year in Japan. More than three-quarters of these occur in the elderly, and most accidents result in

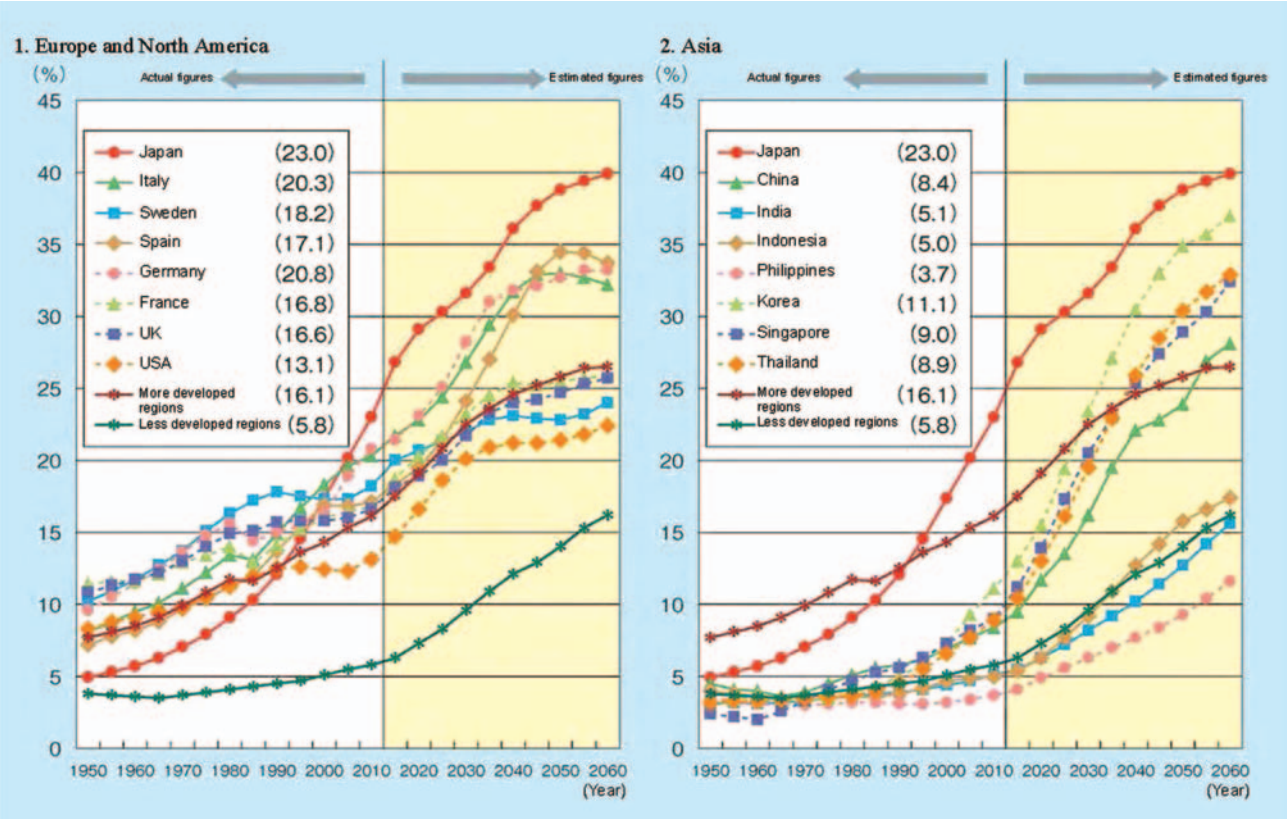

Figure 1. Worldwide Ratio Trend by UN, World Population Prospects: The 2012 Revision (reference: Annual Report on the Aging Society: 2014)

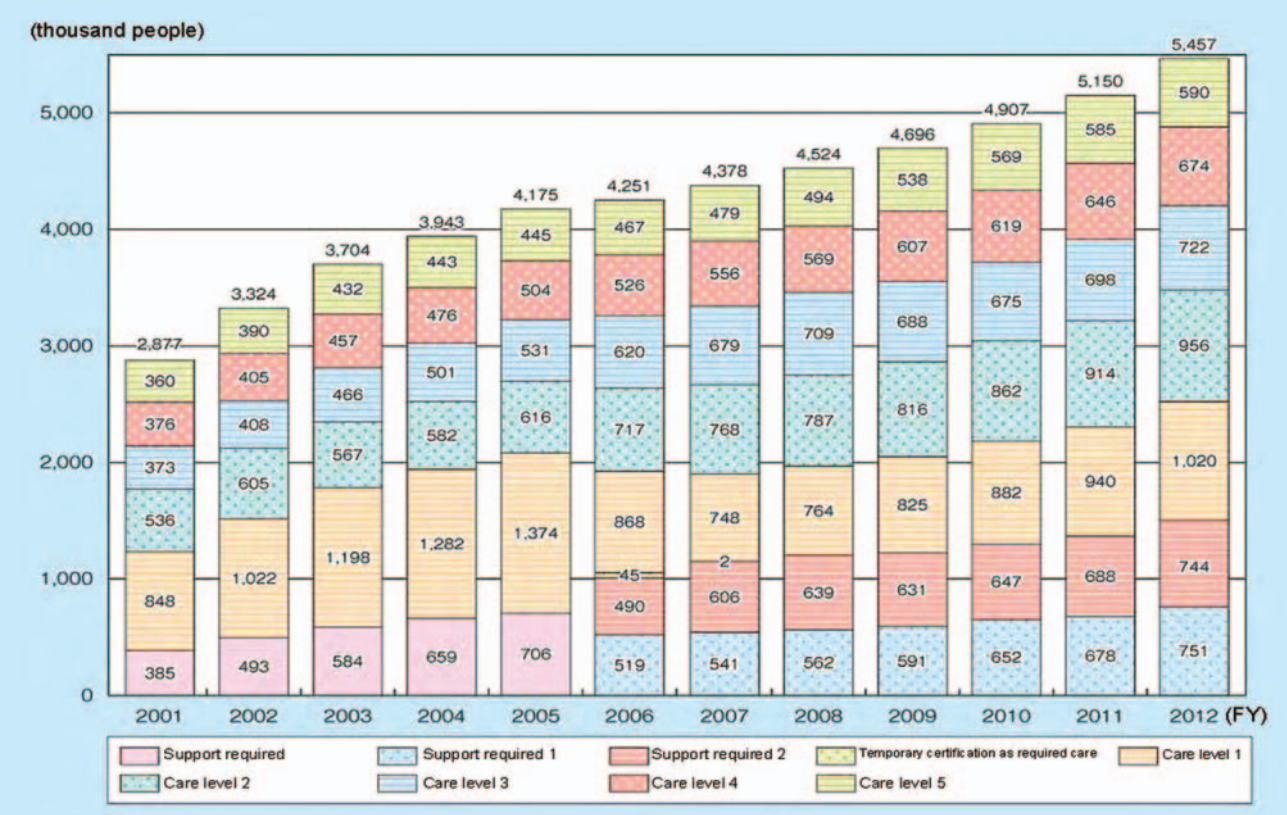

Figure 2. Number of primary insured persons (aged 65 and over) requiring Long-term care or support by care level (reference: Annual Report on the Aging Society: 2014)

death or serious problems[10]. The main foods involved in choking accidents are rice cakes, boiled rice, bread, and rice porridge[10]. Choking accidents in the elderly are also a serious issue in Japan. Pneumonia is the third largest cause of death in Japan, with 122,969 deaths in 2013[11], and it is the largest cause of death among the elderly. Dysphagia rehabilitation is therefore increasingly used in nursing care to prevent aspiration pneumonia and choking accidents.

The need of dental hygienists

The Dental Hygienist Act sets out that dental hygienists provide a range of services including prevention of dental disease, assistance with dental treatment and oral health promotion. The number of dental hygienists in Japan was 116, 299 in 2014, and 90.5% of them were working at dental clinics, 5.1% in hospitals, and 1.8% in municipalities[12]. The need for dental hygienists has recently started to change alongside the demographic changes. Miura reported five new needs for dental professionals, which included home dental care, geriatric dentistry, dysphagia rehabilitation, tissue engineering and regenerative medicine[13].

Nursing care staffs in facilities for the elderly have been reported as wanting support from dental professionals, including provision of information about theory and practice of oral healthcare[14]. The specific issues on which help was sought were deterioration of oral function among service users and lack of any assessment or basic manual on oral healthcare in the facility[14]. Dental professionals, including dental hygienists, are therefore needed to provide this information and support, including assessment and rehabilitation of dysphagia.

Education for prospective dental hygienists

The education system for dental hygienists in Japan has recently been changed from a 2-year course to a 3-year course because of increased professional requirements. The new course now includes training on dysphagia rehabilitation for the elderly[15], and the national examinations for dental hygienists have recently included several questions about this topic.

The School of Oral Health and Welfare was established in 2007 in the Faculty of Dentistry, as the newest school of Tokushima University, and was designed to develop oral healthcare sciences. The policy of the school is to contribute to a healthy long life for everyone by improving and promoting oral healthcare, and to develop specialists and leaders in the education, research and clinical establishments[16]. The curriculum for dental hygienists covers various subjects concerned with the oral health of older and disabled people (see Figure 3). Inter-professional education (IPE) is covered in the first year, and early clinical training is covered in the first and second years. In the third year, there are over 30 hours of relevant courses, with 8 hours of practical training, covering oral health and welfare for the elderly, oral healthcare for the elderly and the disabled, and dysphagia rehabilitation. Prospective dentists and dental hygienists are therefore educated in dysphagia rehabilitation, which is a critical step in helping these professionals fulfill the demands of Japan's super-aged society. Practical training in dysphagia rehabilitation includes how to assess swallowing function with various screening tests, making a rehabilitation plan, indirect training, practice in feeding, and providing oral healthcare for a simulated

Figure 3. Curriculum for dental hygienists at Tokushima University, Faculty of Dentistry, School of Oral Health and Welfare

a: Feeding a simulated blind patient

b: Oral healthcare for a simulated patient with facial paralysis

Figure 4. Practice of feeding and oral healthcare for a simulated patient

patient with facial paralysis (see Figure 4).

Team approach in dysphagia rehabilitation and the role of dental professionals

Dysphagia rehabilitation is performed by multi-disciplinary or trans-disciplinary teams, which may include a doctor, dentist, nurse, speech therapist, dental hygienist, dietitian, radiation technologist, and pharmacologist. The dentist clinically evaluates swallowing and any oral conditions impeding it, devises individual rehabilitation plans and oversees their implementation, and makes any oral devices required. The dental hygienist encourages and assists the establishment of good oral healthcare before and during rehabilitation, and can take responsibility for rehabilitation under the guidance of the lead doctor. Dentists and dental hygienists are therefore expected to take a lead in dysphagia rehabilitation, driving good oral healthcare and rectifying impaired oral function. Medical and dental professionals therefore have to learn to work together in an effective team to provide good medical care.

Tokushima University provides a program on interprofessional education (IPE) to allow professionals to learn about and experience team medical care. The university has three faculties and five schools related to medical and health sciences (medicine, dentistry, pharmacology, nursing and nutrition), and students of all five participate in the IPE program, working in cross-disciplinary groups for a workshop. The IPE program is an effective way for them to learn about each role, how to collaborate with other healthcare professionals, and the role of interactivity in team medical care.

CONCLUSION

There are several roles for dental professionals in dysphagia rehabilitation. Dentists clinically evaluate swallowing and any oral conditions impeding it, devise individual rehabilitation plans and oversee their implementation, and make any oral devices required. Dental hygienists encourage and assist the establishment of good oral healthcare before and during rehabilitation, and can take responsibility for rehabilitation under the guidance of the lead doctor.

Dentists and dental hygienists are expected to lead in dysphagia rehabilitation, driving good oral healthcare and rectifying impaired oral function. Dental professionals therefore have a key role in working with other medical professionals to improve and support eating function for the elderly and the disabled.

ACKNOWLEDGMENT & CONFLICTS OF INTEREST

The author has no conflicts of interest to declare.

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Session X

Oral Cancer in Asia

Global Epidemiology of Oral Cancer: One Disease or Two??

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Tobacco and Alcohol Misuse and Risk of Oral Cancer

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Global Epidemiology of Oral Cancer: One Disease or Two??

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Key words: Oral cancer, oropharyngeal cancer, human papilloma virus (HPV), epidemiology, cancer statistics

ABSTRACT

"Oral cancer" is ill defined and cancer registries may record cancers from many different sites in the head and neck under this single umbrella term. Despite a marked reduction in tobacco use in many parts of the world, oral cancer has continued to increase. Careful analysis of the data however has shown that the increase is mainly seen in younger individuals and in oropharyngeal lesions. Much research has shown that these lesions are associated with HPV infection and provides good evidence that oral and oropharyngeal cancer are separate diseases with distinctive clinicopathological and prognostic features. It is now essential that clinicians, pathologists and epidemiologists are more precise in their definitions of these cancers, since there is clear evidence that they may require different management strategies.

"Oral cancer" is ill-defined and definitions vary across the world. Overall, most agencies use the WHO ICD system (WHO, 2015) which groups malignant neoplasms of the lip, oral cavity and pharynx together under ICD C00 to C14 (Table 1). Cancer registries use this scheme to code neoplasms for epidemiological purposes, but there is variance in which ICD numbers are included. For example Globocan 2002 defines "Oral Cavity Cancer" as C00 to C08, which includes neoplasm of the salivary glands, but these are not normally regarded as "oral cancer". Cancer Research UK (CRUK, 2015) publish oral cancer statistics, but define oral cancer as C00-C06, C09-C10, and C12-C14, which excludes salivary glands, but includes oropharynx and hypopharynx. Almost all statistics include oral cavity and pharynx together as "oral cancer", despite the fact that there is good evidence that cancers at different sites around the upper aerodigestive tract have different causes and different biological behaviours. Our current epidemiological data does not allow us to fully understand the full implications of these differences.

Table 1. ICD (WHO) classification of malignant neoplasms of the lip, oral cavity and pharynx - C00-C14

C00	Malignant neoplasm of lip
C01	Malignant neoplasm of base of tongue
C02	Malignant neoplasm of other and unspecified parts of tongue
C03	Malignant neoplasm of gum
C04	Malignant neoplasm of floor of mouth
C05	Malignant neoplasm of palate
C06	Malignant neoplasm of other and unspecified parts of mouth
C07	Malignant neoplasm of parotid gland
C08	Malignant neoplasm of other and unspecified major salivary glands
C09	Malignant neoplasm of tonsil
C10	Malignant neoplasm of oropharynx
C11	Malignant neoplasm of nasopharynx
C12	Malignant neoplasm of piriform sinus
C13	Malignant neoplasm of hypopharynx
C14	Malignant neoplasm of other and ill-defined sites in the lip, oral cavity and pharynx

(WHO 2015)

Taking a number of specific examples, we can show that in the UK the prevalence of cigarette smoking gradually declined (Hindle et al. 2000a) throughout the 20th century, and now less than 20% of the population use cigarettes. Throughout this period, the incidence of lung cancer fell, but oral cancer continued to increase (Hindle et al. 2000a), leading to speculation that alcohol may play a major role (Hindle et al. 2000b). There is a similar situation in Japan where there has been a gradual decrease in cigarette smoking (Funatogawa et al. 2013) but with no reduction in the incidence of oral cancer. This paradox cannot be easily explained by alcohol consumption alone.

A more careful analysis of the data has shown that the greatest increases in oral cancer incidence have been among younger individuals (Hindle et al. 1996) (Figure 1), and in the oropharynx (Figure 2). In a Swedish population, Hammarstedt et al. (2006) showed a 280% increase in the incidence of tonsillar cancer between 1970 and 2002. Over the same period, the increase in the proportion of cancers infected by HPV rose from 23% to 68%, indicating that HPV played a major role in the increasing incidence of oropharynx cancers. It is now well established that oropharyngeal cancer is associated with infection by high risk human papilloma viruses (HPV) (Gillison et al. 2000; Gillison 2004). Most studies have been undertaken in Europe, USA and Australia, and have suggested that the "HPV epidemic" is confined to western countries. Chaturvedi et al. (2013) evaluated the global incidence patterns of oral and oropharyngeal can-

cers between 1983 and 2002, and showed that the most significant increases in oropharygeal cancers were in the developed countries including USA, Japan, Australia, Canada and Slovakia, and were associated with decreases in oral cancer. In the UK and Denmark they found increases in both cancer sites although oropharyngeal cancers showed a stronger upward trend. Less well developed countries including parts of South America and Asia did not show increases in oropharyngeal cancer. India was unusual in that there was a significant increase in oral cancer and decrease in oropharyngeal cancers. In almost all countries increases in orophayngeal cancers were most prominent in younger age groups (less than 60y) and in men, and were associated with a declining incidence of lung cancer. These results suggest that the increasing incidence of orophayngeal cancer is associated with HPV infection and is independent of tobacco use. The higher prevalence in developed countries is thought to be a reflection of different sexual behaviours, whereby multiple sexual partners and oral sex generally increase the carriage of HPV in the population. In some countries, including India and parts of Europe tobacco use is still highly prevalent and changes in HPV related cancers may be masked and not apparent. However, it needs to be emphasised that HPV is only responsible for up to about 50% of oropharyngeal cancers and that tobacco and alcohol use remain the most important risk factors for both oral and oropharyngeal cancers.

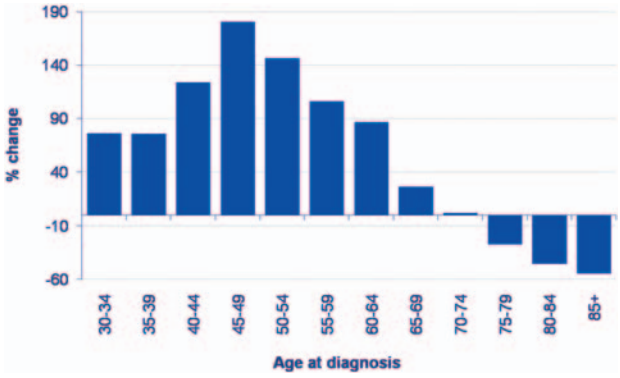

Figure 1. Percentage change in incidence rates for oral cancer in the United Kingdom, for males between 1975 and 2005. The greatest changes have been in the 40-54 year age groups. (Source: CRUK 2015).

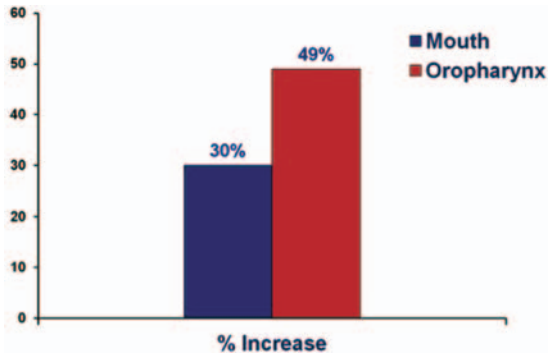

Figure 2. The percentage increase in mouth cancer (ICD 10: C01-C06) and oropharyngeal cancer (ICD10: C09-C10) in England between 1999 and 2009. (Data retrieved from CRUK 2015 and Office for National Statistics, UK (http://www.ons.gov.uk/ons/index.html))

Taken together these studies show quite marked changes in the distribution of head and neck cancers and provide good evidence that oral and oropharyngeal cancers should be regarded as different entities (Figure 3). As well as site distribution the two lesions have distinct clinical and histopathological features and prognosis (Table 2). Oropharyngeal carcinomas are characterized by having a basaloid morphology with no or minimal evidence of keratin formation. For diagnostic purposes the lesions can be shown to stain positively for p16 or to contain active HPV when tested by in-situ hybridisation or PCR (Westra 2015). Although apparently poorly differentiated they have a better response to treatment than oral cancers and have an overall better prognosis. A number of studies are investigating the possibility that HPV positive oropharyngeal cancers can be treated less aggressively than HPV negative lesions. It is important

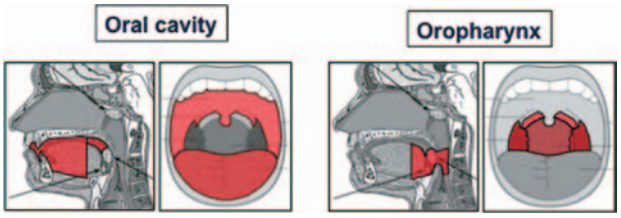

Figure 3. Oral and oropharyngeal cancers have a distinct site distribution and should be regarded as different entities.

Table 2. A summary of the key features of oral and oropharyngeal cancer

Oral cancer	Oropharyngeal cancer	
HPV status	HPV negative	HPV positive
Site	Oral cavity	Tonsil & Oropharynx
Age Group	> 60 years	< 60 years
Gender	M:F = 3:1	M:F = 1:1
Clinical presentation	Early stage (small)	Late stage (large)
Prognosis	Poor response to treatment Poor prognosis < 70% 5 year survival	Good response to treatment Good prognosis > 80% 5 year survival
Histopathology	Squamous, Keratinizing	Basaloid, Non-keratinizing
Causation	Tobacco & Alcohol	HPV; Sexually transmitted?

therefore that these lesions are identified as separate entities and that cancer registries properly record the sites and types of cancer being managed.

In summary, the definition of oral cancer is currently too broad to allow accurate analysis of the different site-specific cancers that may arise in the head and neck. The discovery that oropharyngeal cancer is associated with HPV and that it has unique clinicopathological and prognostic characteristics, is good evidence that there are at least two different disease entities in the head and neck which have previously been defined under the single umbrella of "oral cancer". Going forward it is now essential that clinicians, pathologists and epidemiologists are more precise in their definitions of these diseases, since there is clear evidence that they may require different management strategies. Other specific entities already well defined in the head and neck includes nasopharyngeal and some paranasal undifferentiated carcinomas. Further studies may result in better definitions of cancers at other sites, for example mobile tongue and lip, and it may well be that these should also be regarded as unique clinicopathological entities. Such research will only be possible if we adopt more accurate recording and reporting of cancers by site of origin.

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Tobacco and Alcohol Misuse and Risk of Oral Cancer

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Key words: tobacco, smoking, alcohol, oral cancer, risk factors, life style

ABSTRACT

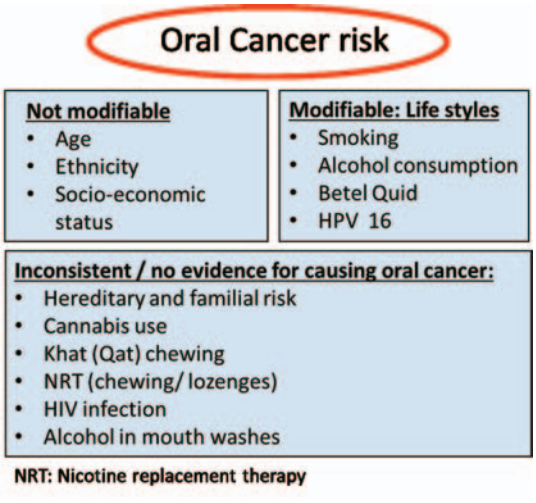
Tobacco smoking is the single largest cause of cancer worldwide, and excess alcohol consumption has been shown to cause cancers of the oral cavity, pharynx, oesophagus and others. Oral cancer is a socio-public health problem in countries where its incidence is high and where ironically medical and dental manpower resources are limited. Primary prevention and early detection are key issues, particularly in South, South-East and East Asian countries. To cope with these circumstances, various international organizations for cancer prevention should work with governments, and national investigators in Asia to develop appropriate educational strategies, media campaigns and screening models and evaluate their effectiveness.

INTRODUCTION

Lip and oral cavity cancer is the 11th most common malignancy in men and 17th in women reported worldwide in the GLOBOCAN 2012[1]. Although the availability of the data varies at the country level, the highest ratio in the world is the South-East Asia region (SEARO), 3rd in the incidence rate and 4th in the mortality rate among all cancer sites in men. The global number of new cases of oral cancers (C00-06, C14) was estimated at 405,318 (age standardized incidence rate, 6.42 for male and 3.27 for female per 100,000), about two-thirds of them arising in developing countries. Higher rates are reported in South Asian, South East Asian and East Asian countries such as in India, Pakistan, Sri Lanka, Taiwan and some pacific islands such as Guam and Papua New Guinea; almost a third of all reported cancers may occur in the mouth or pharynx in these populations. Other high incidence countries include France, Hungary, Brazil, Cuba and Puerto Rico[2]. Oral cavity cancer incidence rates increased in many countries with tobacco epidemics that are currently peaking and declined in areas where tobacco use peaked some decades ago[3]. A recent global data concluded that age-standardized incidence rates (ASIRs) per 100,000 for both sexes in 2013 were higher in developing vs developed countries for lip and oral cavity cancer (7 vs 6)[4].

Risk Factors for Oral and Head and Neck Cancer

Despite good evidence based reports in the global literature[2-4], public awareness about causality of oral cancer is very low the world over. International Agency for Research on Cancer (IARC) through several expert Working Group Meetings has reported on carcinogenic substances that may increase the risk of cancers of the oral cavity and pharynx of humans[5-7]. These include tobacco (both smoked and smokeless), alcohol and betel quid (areca nut) chewing (all listed as Group 1 carcinogens) with or without tobacco, all amounting to lifestyles[8]. Several meta-analyses of published cohort and case-control studies have also confirmed significantly elevated risks of these substances in different population groups[9-15]. Infection with human papilloma virus is an emerging risk factor (particularly for oropharynx; posterior tongue, soft palate, tonsil and anterior pharynx) suggesting a possible link to sexual transmission of this virus from partners who may also be at risk of cervical cancer[16]. Some risk factors are limited to particular pop-



Modified from Warnakulasuriya. (18)

Fig 1. Risk factors with and without any evidence for oral cancer
Factors influencing risk of oral cancer and those with no scientific evidence.

ulations who indulge in specific habits and one such example is matè drinking among people in some Latin American countries[17]. The public often remains concerned about factors that are controversial for causation of oral cancer e.g. spicy foods, mouthwashes, HIV[18], and the health professionals need to be aware of these issues that are best ignored in population approaches to screening and to focus on major risk factors. Fig 1 illustrates causative factors for oral and oropharyngeal cancers, and those with limited or no evidence as to their causative effects.

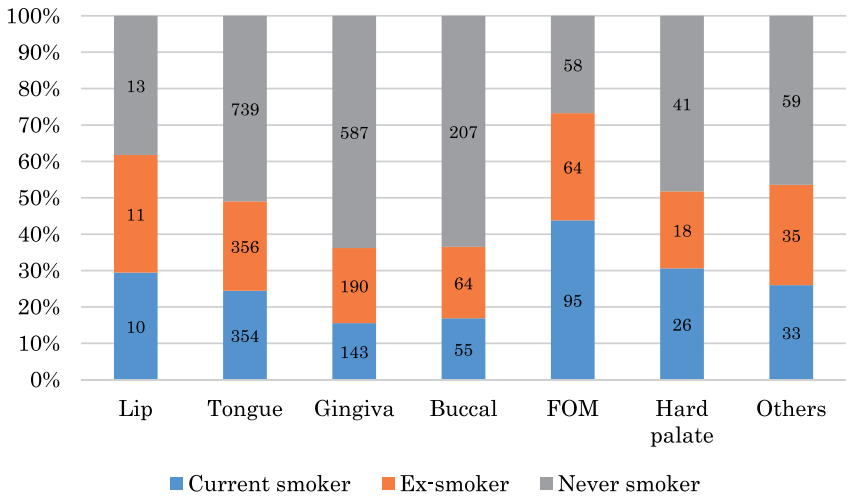
Socioeconomic Status and Oral Cancer

The interaction of smoking and alcohol significantly increases the risk for aero-digestive cancers[19]. Higher rates of oral and head and neck cancer are reported in deprived populations[20] and socioeconomic differences are partially attributable to the population distribution of tobacco smoking and alcohol use in various socioeconomic strata[21]. Poor nutrition, particularly those lacking anti-oxidants may also account for an increased risk for oral and head and neck cancer in deprived societies[22]. Younger people presenting with oral cancer are the exception as a proportion of them come from affluent groups[23]. Any associations of deprivation or dietary factors to risky lifestyles are more likely in older adults. It is therefore clear that a high risk individual for oral cancer can be defined based on three major risk factors (tobacco, alcohol and areca nut) particularly among groups from poor socioeconomic status defined by their level of education, income and employment and also by nutrition. Among younger persons it may not be possible to stereotype who may or may not be at risk.

Tobacco and Alcohol Use and Risk of Oral Cancer in Japan

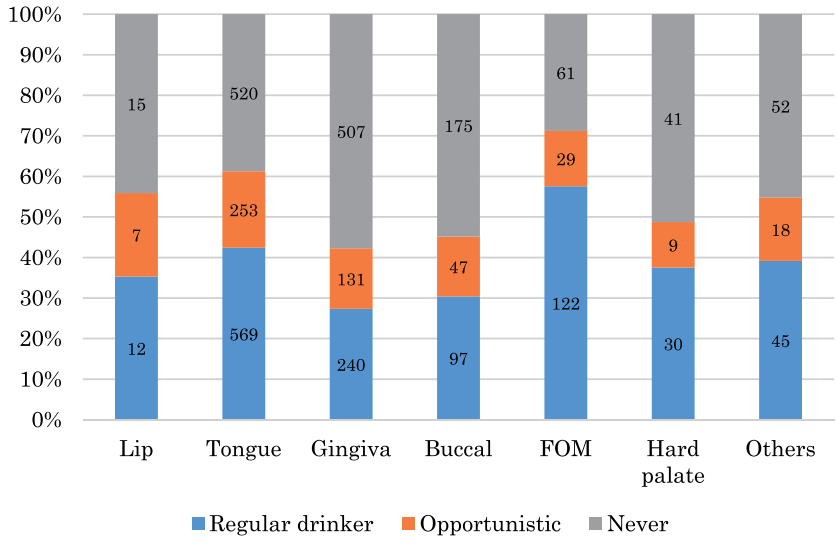
The Japanese Society of Oral and Maxillofacial Surgeons and other 3 societies performed a cross-sectional study on the relationship between tobacco smoking and drinking and oral diseases among Japanese using a questionnaire survey[24]. Among 503 related designated training facilities in Japan, information on the clinical and demographic characteristics were collected from patients with oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD) and other tobacco related oral diseases during a one-year period from 1 January 2012 to 31 December 2012. Results suggested that the frequencies of current smokers with OSCC (n=3,223)/OPMD (n=1,244) were 31.9%/41.0% in males and 8.8%/17.3% in females, respectively. According to the national survey in Japan, average smoking rate was 31.4% in male and 9.0% in female in 2012[25]. The rates of smoking in OSCC (43%) (Fig 2) and OPMD (52%) in the floor of the mouth were highest among all primary sites. On the other hand, gingiva has the lowest rate (16%), implying gum leukoplakias may have a different aetiology. Gum and buccal carcinomas were more common in never-smokers, 64% respectively. The rates of regular drinkers were higher on the floor of the mouth (58%) in oral cancer (not shown) (Fig 3). Gingiva has the lowest rate (27%) in regular drinkers same as the figure of the smoking rate. Radoï et al. performed a large size of population-based case-control study in France, a high-incidence area and indicated that the floor of the mouth was the subsite that was the most commonly affected by the harmful effects of tobacco and alcohol, whereas the gums were less susceptible[26]. Schepman et al. suggested that leukoplakias in the floor of mouth appeared to be significantly more often present in smokers[27]. These results indicate that smoking and drinking habits was strongly associated with oral cancer/precancer in the floor of the mouth, but not on the gingiva.

Recent reports strongly implicate a metabolite of ethanol, acetaldehyde, acting as one of the carcinogens for oesophageal and oral cancer. Importantly, more than



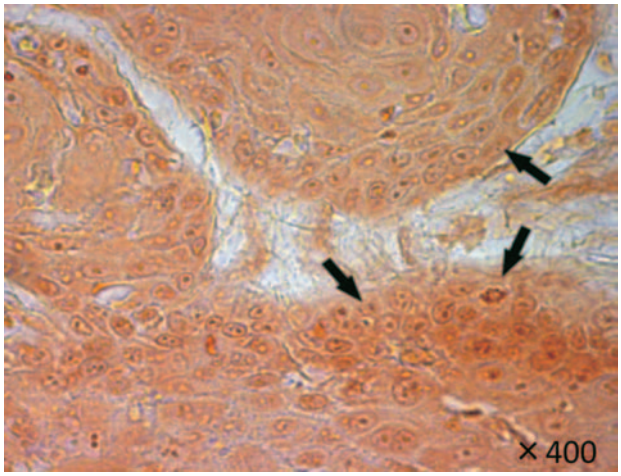
FOM: floor of mouth From Nagao et al. (24)

Fig 2. Smoking rates in oral cancer
For the smoking rate in the current smokes, floor of mouth (FOM) is the highest (43%), followed by hard palate (31%), lip (29%) and others (base of tongue, soft palate and others) (26%). Gingiva has the lowest rate (16%), less than half of that of FOM.



From Nagao et al. (24)

Fig 3. Drinking habits in patients diagnosed with oral cancer
For the drinking rate in the regular drinkers, floor of mouth (FOM) is the highest (58%), followed by tongue (42%), others (39%) and hard palate (38%). Gingiva has the lowest rate (27%), less than half of that of FOM.



From Warnakulasuriya et al. (31)

Fig 4. Acetaldehyde protein adducts in oral cancer tissues in a Japanese male
Representative photomicrograph of acetaldehyde-protein adducts in 47-year- old Japanese alcoholic patient with oral squamous cell carcinoma. Moderate cytoplasmic staining is seen in invading tumor cells.

40% of Japanese people have the inactive form of aldehyde dehydrogenase (ALDH2) leading to accumulation of acetaldehyde that increases the risk for cancer[28]. Acetaldehyde has been found to cause mutations, DNA adduct formation and also inhibit DNA repair[29,30]. The explanation for increased cancer risk associated with increasing alcohol consumption is not clear yet, but we reported that acetaldehyde and lipid peroxidation-derived adducts are formed in oral tissues of alcohol misusers with oral leukoplakia and cancer both in Japanese and British subjects[31] (Fig 4). Further study should investigate and evaluation the amount of acetaldehyde deposited in the oral cavity in alcohol users, as well as to examine oral mucosal tissues in different subsites to confirm any susceptibility of specialised mucosae.

Smoking Cessation and Alcohol Abstinence for Oral Cancer Prevention

Oral health professionals could provide smoking cessation advice at the point of care[32-34] as well as alcohol abstinence advice at minimum cost. Interventions for the improvement of poverty, including welfare and social services provide a new strategy for health promotion[35]. Global oral health inequalities require health media and social initiatives[36]. It is, however, very hard to implement these in terms of health policy. Creating awareness education for oral cancer within the society to improve health literacy involving the pharmaceutical companies, private organizations and governmental agencies must be encouraged.

Based on the outcome of our earlier national survey for oral cancer/precancer[24], the Committee for Tobacco-Free Society of the Japanese Society of Oral and Maxillofacial Surgeons and other 8 Japanese societies are planning to implement a multicentre prospective trial for tobacco cessation intervention in patients with OPMD and other tobacco-related oral diseases in order to achieve lesion control as an intermediate measure and a significant outcome to reduce malignant potential in the OPMD.

CONCLUSION

Oral cancer is a socio-public health problem in countries where its incidence is high and where ironically medical and dental manpower resources are limited. Primary prevention and early detection are key issues, particularly in South, South-East and East Asian countries. To cope with these circumstances, various international organizations for cancer prevention, and overseas

development agencies from developed countries should work with governments, and national investigators in Asia to develop appropriate health education and screening models and evaluate their effectiveness.

ACKNOWLEDGEMENT

The author would like to thank Professor Saman Warnakulasuriya (King's College London) for his contribution by reviewing the manuscript.

No conflict of interest.

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Oral Cancer in Asia

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ABSTRACT

The epidemiology of Oral Cancer in Asia is unique and varies from country to country. This paper aims to present a brief overview of oral cancer status and research activities in Asia. The mixture of low, moderate and high-income Asian countries lead to different needs of each country. Research and programmes for oral cancer in these countries would be in tandem with the countries needs and capabilities ranging from collecting baseline prevalence data to those using advanced technologies towards the development and validation of the biomarkers.

INTRODUCTION

The prevalence of oral cancer is unique in each country in Asia due to the variation in lifestyles and habitual risk factors. The differences in research results maybe a true difference or maybe due to the differences in diagnosis and site of the lesions. Research in oral cancer includes epidemiological research into obtaining baseline data and risk factors of oral cancer for the country. Early detection of oral cancer includes determining level of awareness of oral cancer and creating awareness among the population in particular, for the risk population, conducting screening using the clinical oral examination (COE) as the main screening tool (Kao et al., 2015).

Biomarkers may represent as a type of adjunct to screening and its development may assist in the prediction of certain demographic or clinico-pathologic parameters for example lymph node metastasis while, some may be potential prognostic biomarkers and for the development of therapeutics.

Research activities differ from country to country in Asia dependent on the country's capabilities in terms of funding and research infrastructure. Collaborative research encompasses the research materials and/human resource crossing borders in the move towards achieving one goal of reducing the burden of oral cancer in the region. However, despite all these efforts, the survival rate of patients with oral cancer remained dismal hovering around 50% or lower over the last 4 decades. The low survival rate has been attributed to the high prevalence of late stage cancers of over 60% in many Asian countries. Thus for some countries in Asia, the reasons for late stage cancers includes a delay in diagnosis. Many of these countries needed to have concerted efforts on early detection of oral cancer.

For sometime, India has been cited the highest incidence of oral cancer in the world. However, recent reports showed that Sri Lanka and Pakistan are now ranked at the top. More recent records had identified South Karachi in Pakistan as having the highest rates (Warnakulasuriya, 2009). Many of the oral cancer patients in the Southeast Asian region are betel-quid or areca nut chewers. Other risk factors other than betel-quid, which are associated with oral cancer are tobacco smoking, alcohol drinking with HPV as a debatable risk factor. Some demographic and clinicopathologic parameters have also been shown to be prognostic and/or risk indicators (Krishna et al., 2014). Additionally, an Asian Betel Quid Consortium (ABC) study found that the betel quid chewing rates among men (10.7-43.6%) were significantly higher than women (1.8-34.9%) in Taiwan, Mainland China, Nepal and Sri Lanka. However, the women's rates (29.5-46.8%) were higher than that for men (9.8-12.0%) in Malaysia and Indonesia. This paper aims to give a brief overview of oral cancer status in Asia.

Early Detection of Oral Cancer

Among the reasons for delayed presentation of oral cancers were inadequate knowledge and skills of dental and other health professionals in recognizing early cancer or its precursors. Patients' delay will also lead to them presenting with late stage oral cancer. A study of delayed presentation of oral cancer showed that patients thought that their symptoms are trivial and that the lesion would go away (Rogers et al., 2011). Three studies in India have also shown that while the public are aware of oral cancer and its risk factors, their knowledge about early signs of oral cancer is low and is significantly related to level of education (Elango et al, 2009, Devadiga and Prasad, 2010, Reddy et al., 2012). Elango et al, 2009 conducted a questionnaire survey in a semi-urban area in Kerala where the final participants was 845 participants. Devadiga and Prasad, 2010, and Reddy et al, 2012 conducted their survey at the dental hospital. In Sri Lanka, a study on hospital patients showed that three were awareness of oral cancer and risk habits but 18% were not knowledgeable about the association of risk habits (Ariyawardana, 2005).

In view of the increasing burden of healthcare costs when patients have advance oral cancer; or the fact that many low income countries do not have enough facilities or human resource to manage the oral cancers, there is a need to improve the delivery of knowledge on oral cancer to the public.

When knowledge on oral cancer were compared between the undergraduate dental students' and medical students, a study in Malaysia showed that clinical changes of oral cancer were better identified by the dental students as compared to the medical students (Awan et al, 2013). It is also equally important to train dental and medical professionals to recognize clinical changes of

the oral mucosa. It has also been shown that dentists may also miss oral cancer cases during oral examination (Awan et al, 2013).

While researchers in some Asian countries continues to explore the areas of biomarkers as predictive or therapeutic targets, the final development of a target may require 5-10 years to be completed and ready for clinical trials. Thus, for many low and middle income Asian countries, there is a need to take steps in increasing the awareness of oral cancer among the public. In addition, oral cancer screening whether it is targetted or oppurtunistic are very much needed to ensure early detection of oral cancer and precursor. Efforts to train dental and health professionals in systematic oral examination of the oral mucosa and the neck region need to be enhanced.

Biomarkers for oral cancer

The era of advanced high-throughput technologies of array CGH, gene expression microarray, RNA-sequencing, exome sequencing and protein array has led to a 'boom' in the number of potential biomarkers being reported worldwide. These biomarkers have been shown to have enormous potential for cancer grading and staging. Despite this, only few markers have been integrated into clinical practice. These potential biomarkers need to firstly be validated such that they are able to differentiate between the tumor and normal control tissues, able to predict the clinico-pathological parameters in particular lymph node metastasis and tumor staging and having a prognostic potential through a survival analysis of these oral cancer patients. Having passed the necessary in-vitro and in-vivo validation tests, consideration can be given for them to be biomarkers of clinical trials in cancer therapy such that these biomarkers would be able to differentiate between the malignancy and normal samples. However, especially for validations using immunohistochemistry or PCR on patient samples, the criteria of REporting recommendations for tumor MARKer prognostic studies (REMARK), proposed by the Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics should be strictly adhered to.

Biomarkers for oral cancer maybe to assist in early detection, as an adjunct to diagnosis, prediction and prognosis. Many types of samples can be utilized for biomarker detection, these are biomarkers in saliva, serum and tissue.

Over 100 potential salivary biomarkers for oral cancer detection have been reported in the literature. These studies are mainly based on comparing the levels found in OSCC and non-OSCC normal controls (Cheng et al, 2014). A number of categories of biomarkers have been reported namely, non-organic compunds, peptides, proteins, DNAs, RNAs, mRNAS, microRNAs, long non-coding RNAs, oxidative stress-related molecules, glucocorticoids, metabolomics, glycosylation-related molecules and other molecules for example telomerase. In these salivary biomarker research, a number of challenges need to be considered eg varying collection methods, varying results from one researcher to another of the same marker for example IL-6 and IL-8 where the levels in normal controls differ markedly being indicative of the need to consider the confounders of the different study populations. Research in these areas would require high-end facilities with ample research grants. Thus, only a few such studies are from the Asian region namely from China, Taiwan, Korea and Japan.

Several recent studies on serum biomarkers have been identified but it is not within the scope of this paper to give an exhaustive list of these available biomarkers. However, some very recently reported serum biomarkers from studies in the Asian region included the identification of a serum chemokine (C-X-C motif) ligand 9 (CXCL9), which was found to be associated with tumor progression and treatment outcome in patients with oral cavity squamous cell carcinoma (Chang et al, 2013). IL-8 in serum was also identified and may be involved in poor clinical outcomes via generation of CD163-positive M2 macrophages. These factors was thought to have prognostic value in patients with resectable OSCSS in addition to N status (Fujita et al 2014). Four candidate biomarkers from serum proteins namely gelsolin, fibronectin, angiotensinogen, and haptoglobin which may be able to predict/detect the presence of lymph node metastasis in oral cancer patients was also reported by Chai et al, 2014. In 2015, Xu et al. showed that high serum miR-483-5p expression was an independent of prognostic factor for OSCC and further suggested this may be a novel diagnostic and prognostic biomarker for OSCC.

Selected tissue biomarkers with reports originating from Asia include Calreticulin (CRT) an endoplasmic reticulum-resident protein in oral cancer (Chiang et al., 2013). CRT is a potential biomarker and may contribute to the malignant phenotypes of OSCC cells. MMP-9 as a potential tissue biomarker for carcinoma of oral cavity has recently been reported as a research findings from a study in eastern India (Nanda et al., 2014). Another recently reported study by the International Cancer Genome Consortium (2013) based on tissue samples reveals molecular subtypes with distinctive mutational profiles such as patients predominantly harbouring mutations in CASP8 with or without mutations in FAT1. In some some molecular subgroups, the mean duration of disease-free survival is significantly elevated. Among the tissue biomarkers reported by researchers in Japan, a recent study data indicated that Angiopoietin-like 3 ANGPTL3 may play a role in OSCCs via MAPK signaling cascades, making it a potentially useful diagnostic/therapeutic target for use in patients with OSCC. (Koyama et al 2015).

A significant association between the amplification of EIF3E gene with non-betel quid chewers and the negative lymph node status were shown in Malaysian oral cancer patients. The results suggest that EIF3E may have a role in the carcinogenesis of OSCC in non-betel quid chewers (Yong et al 2014). A recent literature reporting on studies of Malaysian oral cancer patients showed that co-expression of TWIST1 and ZEB2 in OSCC is associated with poor survival (Kong et al 2013). Additionally, dysregulation of miR-31 and miR-375 expression is associated with clinical outcomes in OSCC of Malaysian patients (Siow et al 2014) and MMP13 is associated with clinical outcomes and poor prognosis in OSCC (Vincent-Chong et al 2013). In another Malaysian study, a significant cor-

relation between LOH/MSI at 1q21.3 with clinical outcomes and downregulation of CRNN gene. Thus, the CRNN gene could be considered as a prognostic marker of OSCC (Salahshourofar et al., 2015).

Development of therapeutics and personalized medicine

The biomarker findings open new avenues for biological characterization and exploration of therapies. Based on the standard methodologies in bringing biomarkers to be useful at the clinical level, the literature on studies on biomarker development from Asian researchers in the last 5 years were briefly reviewed and revealed a number reports suggesting potential therapeutic targets.

Among the potential targets, CXC chemokine receptor (CXCR4) may be a useful target molecule for the treatment of OSCC (Hong et al, 2009 and Yu et al., 2012). Hong et al.'s (2009) study showed that knockdown of CXCR4 in OSCC cell lines reduced cancer cell proliferation, invasion and migration activity. The result suggests that downregulation of CXCR4 induces anti-proliferative and anti-invasive effects in OSCC. Similarly, Yu et al's (2012) study provided new evidence of CXCR4 as a potential target where his study demonstrated that RNA interference-mediated knockdown of CXCR4 in highly aggressive tumor cells (Tca8113 and SCC-9) significantly inhibits the proliferation of the two cell lines in vitro and in vivo. More than 1,500 genes involved in cell cycle, apoptosis, and multiple signaling pathways showed altered expression. These results provide new evidence of CXCR4 as a promising tumor gene therapeutic target.

Annexin (ANXA10) (Shimizu et al., 2012) has also been reported as potential therapeutic targets for the development of new treatments for OSCC. Silencing of ANXA10 by shRNA inhibit the cancer cell proliferation activity through the extracellular regulated kinase (ERK) and cell-cycle pathway. Similarly, silencing of Fibroblasts Activation Protein (FAP) gene by shRNA inhibit the cancer cell proliferation, migration, and invasion via the inactivation PTEN/PI3K/AKT and Ras-ERK pathways. FAP also inhibits the tumor growth and metastasis in xenograft model. Thus, FAP also show promise as anticancer targets (Wang et al., 2014). LIM and SH3 protein 1 (LASP-1) (Shimizu et al., 2013) also inhibits the cancer cell proliferation activity through the cell cycle pathway, suppressing tumor growth by shRNA that targeted LASP-1.

Additionally, Melanoma Antigen Gene Type II (MAGED4B) (Cheong et al, 2012) and G-Protein Subunit Alpha-12 (GNA12) (Cheong et al., 2014) are 2 potential targets being suggested by Malaysian researchers. Overexpression of MAGED4B can promote cancer cell proliferation, with evasion towards apoptosis signal and migration activity. Inhibiting GNA12 expression by shRNA was shown to reduce the cancer cell migration and invasion in vitro. Also, inhibition of GNA12 signalling inhibited GNA12-mediated RhoA activation, resulting in reduced lymph node metastasis in an orthotopic xenograft mouse model. Taiwanese researchers reported that Matriptase and Carbonic anhydrase IX (CAIX) have promising roles for OSCC therapy (Chen et al., 2013 and Yang et al., 2015). Matriptase was shown to also inhibit the cancer cell invasion and migration activity by shRNA treatment while CAIX inhibits the cancer cell migration activity by siRNA treatment.

Thus far, there are yet no reports in the literature of any of these reaching the phase 1 clinical trial stage.

Standardizing criteria in diagnosis of oral cancer types

Criteria in diagnosing the different types of oral cancers mostly follow the WHO criteria (2005). However, varied clinical and pathologic profiles of these lesions may lead to differences in the interpretation of these WHO criteria. Thus in the field of biomarker studies or studies on adjunctive tools, in order to make results comparable within and across countries, efforts towards standardization of these criteria are needed.

As an example, oral squamous cell carcinoma (OSCC) makes up more than 90% of oral cancer (Ref). However, in interpreting the criteria from the WHO classification (2005), variations may also exist since OSCC can be divided into 6 types namely, conventional OSCC, verrucous carcinoma, papillary squamous cell carcinoma (PSCC), variants of squamous cell carcinoma such as spindle, basaloid, adenosquamous and acantholytic types. Whilst the latter 4 variants of OSCC are uncommon or rare and have specific features with its related prognosis, the verrucopapillary variants seemed to be an area of controversy as these may be diagnosed interchangeably with conventional OSCC which exhibits verrucopillary features. The true oral PSCC is supposed to be the counterpart of the laryngeal PSCC, which is associated with HPV (Lewis 2011). However, majority of OSCC are HPV negative for example in Malaysia, more than 90% of 215 cases of OSCC examined are HPV negative (unpublished data). A look through the archives of the Malaysian verrucopapilary cases from year 2000, revealed that most of these cases with papillary features do not fulfill the criteria of PSCC except for 1 case (Zain et al., 2013). Thus, there is a need to recognize these types as the conventional OSCC with papillary features. In relation to studies in identifying biomarkers, will these 2 types have different genetic make-up? Would these then lead to different survival rates? Another area of concern is the diagnosis of Verrucous Carcinoma, which is supposed to have a good prognosis with no cervical node metastasis, but many have reported otherwise due to the use of non-strict criteria for its diagnosis or the non-detection of conventional OSCC foci within the other parts of the Verrucous Carcinoma. These diagnostic challenges needed to be addressed and selection of types of OSCC for many of the molecular based studies in Asia need to be standardized to ensure an accurate representation of study findings.

CONCLUSION

Research in oral cancer may need to be prioritized according to the needs and capability of each country. Collaborative efforts between countries with no or minimal facilities but ample study materials and those with state of art facilities with less study materials would be able to intensify molecular based studies towards biomarkers and therapeutic targets for personalize medicine. However, it is now timely to re-look at the Asian

oral cancers and its related data such that accurate subclassification especially of the spectrum of the oral squamous cell carcinoma with verrucopapillary. In the meantime, efforts for early detection of oral cancer need to be enhanced through networking between Asian specialists as well as a move to assist these countries to have more trained personnel.

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ACKNOWLEDGEMENTS

My gratitude to the staff of the Oral Cancer Research and Coordinating Centre, Faculty of Dentistry, University of Malaya in particular: Wan Maria Nabillah, Vincent Chong, Wong Gou Rean and Suzanne Edgar for their assistance.

Areca/betel Quid and Oral Cancer Epidemiology in Asian Pacific Region

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ABSTRACT

Oral cancer is a common malignancy in India and many other countries of South-East Asia and Asia Pacific. Areca/betel quid chewing, with and without tobacco, was considered as having sufficient evidence of carcinogenicity based on the 37th and 85th volumes of the IARC Monographs. Since then, new reports on additional clarification of areca/betel quid composition were subsequently published. The risk on oral cancer from betel quid without tobacco added was further strengthen by two meta-analyses. Futuer research agenda may consider various types or ways of preparation for using areca nut, since the risk from Taiwan (RR=10.98, 95% CI=4.86~24.84), where betel quid without tobacco is used, was much higher than the pooled estimates from India subcontinent (RR=2.41, 95% CI=1.82~3.19 in users of betel quid without tobacco, and RR=8.47, 95% CI=6.49~11.05 for users of betel quid with tobacco). In terms of priorities for action, the results of population attributable fraction (PAF%=53.7% in Taiwan; PAF%=49.5% in India subcontinent) strongly support the need for betel quid chewing intervention. Furthermore, since the study of interaction accounted for 72.6% of risk effect, any intervention on the areca/betel quid chewing habit toward oral cancer prevention should also consider concurrent tobacco or cigarette smoking and alcohol drinking.

INTRODUCTION

According to GLOBOCAN 2012[1], the estimated number of new lip and oral cavity cancer patients was 300,373 worldwide. Among them, 168,850 (56.2%) cases were from Asia, and 77,003 (25.6%) cases were from India. While in most regions of the world, the leading cancers are those of the lung, breast, prostate and colorectum, in India and many other countries of South-East Asia or Asia Pacific, oral cancer is still a common malignancy. Areca/betel quid chewing, with and without tobacco, was considered as having sufficient evidence of carcinogenicity from the 37th and 85th volumes of the International Agency for Research on Cancer (IARC) Monographs[2,3]. The 85th volume of IARC Monograph provides an extensive review on composition, consumption and risk to oral cancer for areca/betel quid from epidemiological studies published in English. Additional reports on composition of areca/betel quid were later published for countries, including Nepal[4], Vietnam[5], Micronesia[6] and the Solomon islands[7].

In terms of investigation on oral cancer risk from "betel quid without added tobacco" and "betel quid with added tobacco", two meta-analysis reports[8,9] were published in 2014. While majority of areca/betel quid chewers were also concurrently with smoking or drinking habits, the assessment of interaction effect from chewing/smoking/drinking is also of interest[10].

Areca/Betel Quid Epidemiology in Asian Pacific Region

Areca/betel quid chewing is a prevalent habit in many Southeast Asian and Asia Pacific communities. It is chewed or placed in mouth for many reasons, including its psycho-stimulating effect, as well as a social and cultural practice[3]. Areca/betel quid has been considered the fourth most universally consumed substance after tobacco, alcohol and caffeine[11]. It is widely used by at least 10% of the worlds' population (600 million persons) globally.

The term of "betel quid" should always be used with caution. It generally consists of areca nut (from the *Areca catechu* tree), betel leaf (from the *Piper betle* L. vine) and slaked lime (calcium hydroxide) with other local ingredients. In many communities, tobacco is often added to the quid. The term of "betel nut" is not botanically correct and should be avoided in the scientific literatures.

The IARC monograph[3] extensively described different compositions in areca/betel quid as well as and consumption in various areas. In brief, areca nut is the seed of the fruit of the oriental palm *Areca catechu*, which is the basic ingredient in chewing quid. In different geographical areas, areca/betel quid was used or prepared in different forms including unripe/ripe, whole/sliced/chopped, raw/roasted/sun dried, boiled/soaked and fermented. Betel leaf is the most common accompaniment for chewing areca nut globally. It is the leaf of *Piper betle* L. Other parts of the vine such as the stem, inflorescence (also called flower or pods) or catkins are also consumed with areca nut. Consumption of the inflorescence is common in Melanesia and in parts of Taiwan, and it is often added to the quid for its aromatic flavor. Slaked lime (calcium hydroxide) is generally smeared on betel leaf and combined with areca/betel quid. In coastal areas, it is obtained by heating the covering of shell fish (sea shells) or is harvested from corals. In central parts of a country, it is quarried from limestone. Tobacco is often added to the quid mixture (India, Pakistan and Bangladesh) whereas it is never added to the quid in China, Taiwan or Papua New Guinea. Chewing tobacco in the Indian subcontinent is prepared from sun-dried and partly fermented, coarsely cut leaves of *Nicotiana rustica* and *Nicotiana tabacum* without further processing. Sometimes tobacco is powdered and combined with molasses or boiled before use. Other ingredients include catechu (a reddish-brown substance smeared on the betel Leaf), spices

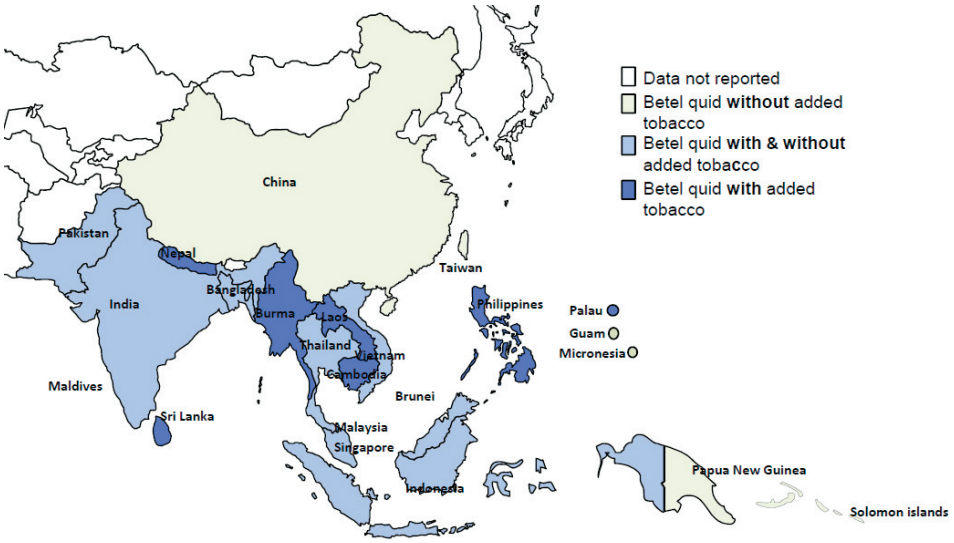

Figure 1. Betel quid with or without added tobacco in different countries

(cloves, cardamom or aniseed), sweeteners (coconut or dried dates) and essences (rose essence, menthol, mint or rose petals).

There are also areca nut-based industrial packaged products available in several countries, mainly *gutka* and *pan masala*. *Gutka* is a dry, relatively nonperishable commercial preparation containing areca nut, slaked lime, catechu, condiments and powdered tobacco. The same mixture without tobacco is called *pan masala*. Both *gutka* and *pan masala* do not contain betel leaves, and are sold in foil packets (sachets) and tins which can be stored and carried conveniently. *Pan masala* and *gutka* are very popular in urban areas of India and Pakistan, especially among adolescents. They are mainly produced in India and exported to over 30 countries.

In terms of regional consumption of areca/betel quid, in India, while both betel quid with and without added tobacco has been used, majority of chewers used tobacco added quid[3]. Several surveys have been conducted in adults and adolescents for rural and urban communities. The prevalence rates appear to be higher in rural adults (around 20%~51%) then in urban adults (around 5%~38%), and be higher in men then in women. Prevalence rates on students and adolescents are also available, and *pan masala* and *gutka* are commonly chewed by children and adolescents.

It was estimated that about 10% of the population in Taiwan, chew betel quid[3]. The ingredients of a quid vary with the areas and ethnic groups. Quid in Taiwan can be classified into three types: lao-hwa quid, betel quid and stem quid. Lao-hwa quid prevails in urban districts, and is prepared by inserting a piece of inflorescence of *Piper betle* L. with red lime paste (slaked lime and some local flavoring) into an unripe areca nut. The second most popular quid is betel quid made by wrapping an unripe areca nut and white slaked lime paste in a piece of betel leaf, and is popular in urban and indigenous areas. The third type of quid, stem quid, is similar to lao-hwa quid except that the piece of inflorescence of *Piper betle* L. is replaced by a piece of stem of *Piper betle* L. The use of stem quid is rare and only seen in southern parts of indigenous areas[12]. Tobacco is never added to any forms of quid in Taiwan.

In Malaysia, betel quid was prepared in different forms according to ethnic groups. The betel quid used by Indians consisted of young betel leaf, slaked stone lime, tobacco and powdered or sliced, dried areca nut, with or without tobacco. Among the Malays, a betel quid consists of a more mature betel leaf, *gambir*, slaked stone lime and fresh areca nut without tobacco. Chewing habit appeared to be more prevalent among women (76.3% of the chewers were women and 23.7% were men)[3].

The areca/betel quid chewing habit is mainly concentrated in the southern provinces of Mainland China[13]. Dried areca nuts, including the husk, are processed as industrially packaged products, which were processed by marinating the halved fruits with different flavored substances including slaked lime. The final products for chewing are half fruits, including the husk in a dried state. Tobacco is never added. On the Hainan Island, the fruit is chewed fresh with slaked lime, wrapped in a betel leaf.

Since the IARC monograph in 2004, additional reports on composition of areca/betel quid were later published for countries, including Nepal[4], Vietnam[5], Micronesia[6] and the Solomon islands[7]. Whether the areca/betel chewing quid contains tobacco or not was all summarized in figure 1 together with regions described in the monograph.

Risk of Areca/betel Quid to Oral Cancer

Two major forms of areca/betel quid was classified by the IARC as "betel quid without added tobacco" and "betel quid with added tobacco". In the 37th volume of the IARC monographs[2], it was reported that sufficient evidence of carcinogenicity was found for betel quid with tobacco, but not for betel quid without tobacco. Since then, many studies have been conducted to investigate effects with or without tobacco. Especially, studies from areas which tobacco is never added to the quid as well as studies from India and Pakistan which effects of both groups were separately analyzed. The 85th IARC mono-

graph consists a thorough review of literatures on their risks to oral cancer. Recently, two meta-analysis reports[8,9] have been conducted to investigate the risk of betel quid with or without tobacco to oral cancer.

A meta-analysis[9] of 15 case-control studies (4,553 cases; 8,632 controls) and 4 cohort studies (15,342) showed that chewing quid with tobacco is significantly and independently associated with an increased risk of squamous-cell carcinoma of the oral cavity (case-control studies: OR=7.46, 95% CI=5.86~9.50, and cohort studies: RR=5.48, 95% CI=2.56~11.71). In addition, the 15 case-control studies has shown betel quid without tobacco to have an independent positive association with oral cancer, with OR=2.82 (95% CI=2.35~3.40). Presumably, due to the carcinogenicity of areca nut, betel quid without tobacco are also strong and independent risk factor for oral cancer.

From a meta-analysis of 50 publications[8] on the investigation of risk to oral/oropharyngeal cancer, the relative risk (RR) for oral cavity cancer was 2.41 (95% CI=1.82~3.19; 13 studies) in users of betel quid without tobacco, and was 8.47 (95% CI=6.49~11.05; 25 studies) for users of betel quid with tobacco. Similarly, both groups have shown significant associations with oral cavity cancer. However, in Taiwan, where tobacco is never added to the quid, the RR was 10.98 (95% CI=4.86~ 24.84; 13 studies). The magnitude of RR was much higher in Taiwan. It is speculated that a larger daily amount was consumed, or the unripe fruit was used.

In Indian subcontinent, the RR was much higher in women (RR=14.56; 95% CI=7.63~27.76) than in men. Dose-response analyses also demonstrated that the risk of oral/oropharyngeal cancer increased with increasing daily amount and duration (years) of chewing areca/betel quid in India and Taiwan. Around half of oral cancers in these countries could be prevented if people stopped their chewing habits (population attributable fraction: PAF%=53.7% in Taiwan; PAF%=49.5% in India subcontinent).

While majority of areca/betel quid chewers were also concurrently with smoking or drinking habits, the assessment of interaction effect from chewing-smoking-drinking is also of interest. One report[10] focused on the magnitude of the smoking-drinking-chewing interaction effect through the pooled Relative Excess Risk due to Interaction (RERI, excess risk in smoking-drinking-chewing exposed individuals with respect to the risk expected from the addition of the three individual risks of smoking, drinking and chewing). The pooled ORs (14 studies) for smoking, drinking, chewing, smoking-drinking-chewing, respectively were 3.6 (95% CI=1.9~7.0), 2.2 (95% CI=1.6~3.0), 7.9 (95% CI=6.7~9.3), 40.1 (95% CI=35.1~45.8). The pooled RERI was 28.4 (95% CI=22.9~33.7). Among smoking-drinking chewing subjects, the individual effects accounted for 6.7% from smoking, 3.1% from drinking, 17.7% from chewing of the risk, while the interaction effect accounted for the remaining 72.6%. The study results suggested that effective oral cancer control policies must consider concurrent tobacco smoking, alcohol drinking, betel quid chewing usages as a unique risk factor for oral cancer.

CONCLUSION

Since the 85th volume of the IARC Monographs[3] was published, many of focus has centered on the clarification of the composition of areca/betel quid in countries with chewing habits as well as on the strengthening of oral cancer risk from betel quid without added tobacco. Further research agenda may consider various types or ways of preparation for using areca nut in quid, since the risk from Taiwan, where betel quid without tobacco is used, was much higher than the pooled estimates from India subcontinent.

In terms of priorities for action, the results of population attributable fraction (PAF%=53.7% in Taiwan; PAF%=49.5% in India subcontinent) strongly support the need for betel quid chewing intervention. Furthermore, since the study of interaction accounted for 72.6% of risk effect, any intervention on the areca/betel quid chewing habit toward oral cancer prevention should also consider concurrent tobacco or cigarette smoking and alcohol drinking.

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Special Lecture

Special lecture I

Patient Simulation and Advanced Clinical Education with SIMROID

J. Morita MFG. CORP.
D.L. Sleeman

Special lecture II

"DENTAPAC KOKORO"
—Epochal Solutions to Overcome Barriers to Dental Care
Subject 1: Next Generation Modular Dental Treatment System

THE YOSHIDA DENTAL MFG. CO., LTD.
K. Kuroda

Special lecture III

"DENTAPAC KOKORO"
—Epochal Solutions to Overcome Barriers to Dental Care
Subject 2: Development of Dental Equipment and Materials for Home-visiting Dental Treatment

GC CORPORATION
F. Fusejima

Special lecture IV

The Evaluation of Human Pluripotent Stem Cell Culture
by Advanced Cell Morphological Analysis

National Institutes of Biomedical Innovation
M. Kusuda Furue

Patient Simulation and Advanced Clinical Education with SIMROID

D.L. Sleeman

International Sales, J. Morita MFG. CORP.

BACKGROUND

Until recently, practical dental education has been performed using sophisticated phantoms and mannequins which allow educational institutes to objectively evaluate technical skills. When it comes to clinical workflow and patient treatment, this must be done with real patients who may present with vastly different treatment needs making the objective evaluation of a student's performance problematic. A system that combines the best of training phantoms with the interactive capabilities is needed to allow the student to practice necessary skill and workflows prior to working with an actual patient. That system is SIMROID.

PRESENTATION

In our presentation, we will introduce the motivation behind this innovative project and introduce you to the people and institutions who collaborated together to achieve this ambitious educational tool.

We will discuss the current gaps in the education system and the underlying motivation that prompted the Nippon Dental University to contact Morita and launch what will eventually become the future of dental education.

We will introduce our audience to the SIMROID dental education system and highlight not only what sets this system apart from the current education tools available but also show what makes the SIMROID uniquely suited to modern objective education. From there, we have an overview of what the patient simulation robot can actually do. We will highlight the wide range of treatments that can be simulated and show how many more are available to students and educators beyond standard phantoms.

We will conclude with some feedback from our dental students who are currently training with SIMROIDs in their schools and share with you their thoughts and reactions to this enhanced education program.

CONCLUSION

Through our presentation and subsequent discussions and demonstration, we hope that the audience will get a good sense of the potential of such a patient simulation system and that they can see the impact training with a SIMROID will have on patient care and ensuring new dentists are better prepared for the realities of working with real patients in a real clinical setting.

"DENTAPAC KOKORO" —Epochal Solutions to Overcome Barriers to Dental Care Subject 1: Next Generation Modular Dental Treatment System

K. Kuroda

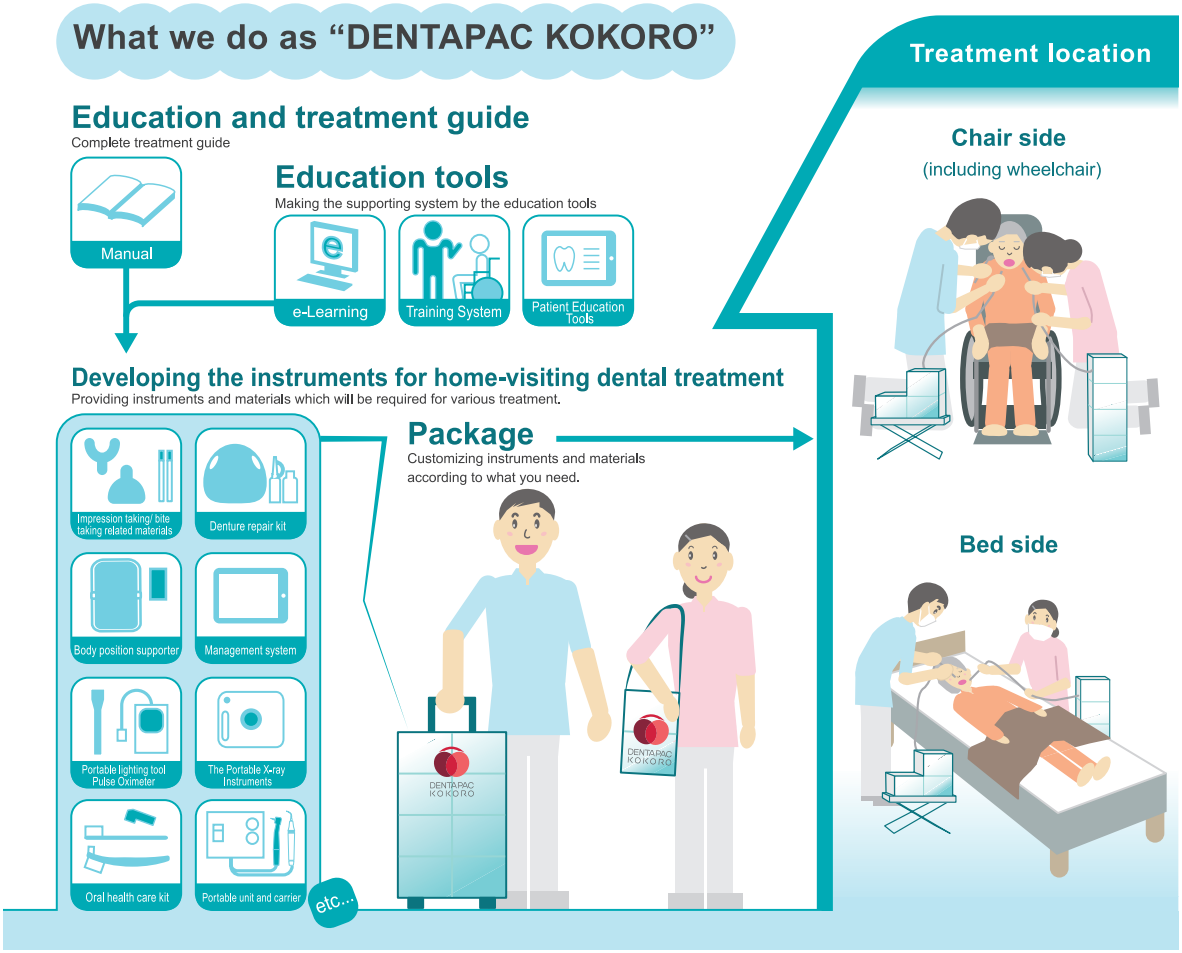
Division Director, International Business Div., THE YOSHIDA DENTAL MFG. CO., LTD.

In Japan, a country of super aging society, there are approximately 6 million elderlies who require nursing care from the others. 90% of functionally dependent elderly is said to be in need of some type of dental treatment, however, the reality is, only 30% of those have been able to receive necessary treatment.

One of the reasons that attribute the aforementioned is the physical as well as psychological stress that doctors experience in providing dental care due to the insufficient treatment tools required for home-visiting dental care.

Under the circumstances, The Japan Dental Association (JDA), the Japan Association for Dental Science (JADS), and the Japan Dental Trade Association (JDTA) have been promoting the development of the portable home-visiting dental treatment system, and in response to a request from the Ministry of Economy, Trade and Industry, the project was actualized by a consortium including 10 member companies of the Japan Dental Trade Association.

As the deliverable, we have "DENTAPAC KOKORO" the modular-designed portable dental delivery unit, which we will introduce in this presentation.



"DENTAPAC KOKORO" —Epochal Solutions to Overcome Barriers to Dental Care Subject 2: Development of Dental Equipment and Materials for Home-visiting Dental Treatment

F. Fusejima

Deputy General Manager, Research & Development Dept., GC CORPORATION

The background : In 2008, "New medical equipment and medical technology industry vision" was formulated by the Ministry of Health, Labor and Welfare. In the article, there is a "the propulsion of home-visiting dental treatment and the development of portable dental instruments for the maintenance of dental health". We chose three theme of development from eight theme for portable dental instruments and started to development.

• Sub Theme 5 "Kit for impression taking/bite taking related materials"

Target : Development of impression tray, impression materials and bite wax for efficient and simple denture fabrication during home-visiting dental treatment.
Result : On July 2010, consortium consisted of J.D.A., J.A.D.S. and J.D.T.A was established and started development. On July 2014, kit of "Impression taking/bite taking-related materials" was launched.

• Sub Theme 6 "Kit for Denture repairing"

Target : Development of self-polymerization resin for efficient and simple denture repair during home-visiting dental treatment.
Result : Consortium was established same as above and started development. On July 2014, "Denture repair kit" was launched

• Sub Theme 7 "Kit for Oral health care"

Target : Development of disposable brush for denture cleaning and oral care brush for oral cleaning for easy denture cleaning during home-visiting dental treatment.
Result : Consortium was established same as above and started development. On July 2014, "Oral health care kit" was launched.

The Evaluation of Human Pluripotent Stem Cell Culture by Advanced Cell Morphological Analysis

M. Kusuda Furue

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Human pluripotent stem cells (hPSCs), including embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) can proliferate virtually infinitely and differentiate into almost all cell types. These hPSCs could be a promising new tool both for cell-based regenerative medicine and pharmaceutical research, including drug efficacy and toxicity screening tests. However, it remains challenging to maintain hPSCs in an undifferentiated state because of their differentiation potential and/or genetic instability. Therefore, routine characterization of hPSCs using several standard criteria, such as cell growth, marker expression, or in vitro differentiation, is recommended. In practice, observation under phase contrast microscope is used to continuously determine the health condition of hPSCs. When hPSCs are cultured under proper conditions, they form tight, compact and rounded colonies. However, epithelial-mesenchymal transition (EMT) occurs at the peripheral of the hPSC colonies where hPSCs differentiate spontaneously. Under improper conditions, hPSCs tend to execute apoptosis or differentiate, and dynamically change their cell morphology. Culture-adapted" cells with karyotypic changes tend to have an increased growth rate or reduced apoptosis. The criterion for evaluating cell morphology is not quantitative. Recently, we have developed a non-invasive method to determine the growth of hPSCs by analyzing morphologies of live, non-labeled hiPSCs in culture without damaging these cells. When the culture conditions become improper, the change in cell morphology could be detected by analysis of the phase-contrast images. This method provides real-time information on the growth and quality of hPSCs without damaging or wasting cells and could be useful both for basic research using hPSCs and for cell processing for hPSC-based therapy.

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Poster Session

A-1~A-9: Dental Education
B-1~B-28: Frontiers of Biological Science in Dentistry
C-1~C-6: Latest Trends in BioDental Engineering
D-1~D-39: Oral Health and Clinical Treatments

■*Airlangga University, Indonesia*

■*B P Koirala Institute of Health Science, Nepal*

■*Catholic University of Pusan, Korea*

■*Chulalongkorn University, Thailand*

■*International Medical University (IMU), Malaysia*

■*Kaohsiung Medical University, Taiwan*

■*Khon Kaen University, Thailand*

■*Kyungpook National University, Korea*

■*Mahidol University, Thailand*

■*National Hospital of Odonto-Stomatology in Ho Chi Minh City, Viet Nam*

■*Padjadjaran University, Indonesia*

■*Prince of Songkla University, Thailand*

■*University of Malaya, Malaysia*

■*University of Medicine and Pharmacy, Ho Chi Minh City, VietNam*

■*University of Peradeniya, Sri Lanka*

■*Wonkwang University, Korea*

■*Hiroshima University, Japan*

■*Stomatological Hospital of Tianjin Medical University, China*

A-1 (01-1) Alteration of Preschool' Personal Hygiene Behavior: Mouth Hygiene by Health Education with Puzzle

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INTRODUCTION : Personal hygiene behaviour such as skin hygiene, mouth hygiene, and hair hygiene is essential for children. In Indonesia, most of children have problems in mouth hygiene. This caused by lack of fasilities in personal hygiene's education which cause less attention to personal hygiene's behavior in children. The function of health education is to change someone's behavior towards personal health. This study was aimed to examine the effect of health education with puzzle to increase preschool's personal hygiene: mouth hiygiene behavior at Aisyiyah Bustanul Athfal II kindergarten, Kedurus, Sidoarjo.

METHODS : Design of this study was Quasy Experiment. The population were students B class at Aisyiyah Bustanul Athfal II kindergarten, Kedurus, Sidoarjo. The samples were taken by simple random sampling technique with the number thirty respondents. The datas were collected by using questionnaire and observation sheet, and analyzed by using Wilcoxon Signed Rank Test and Mann Whitney U Test with significance level of $\alpha \leq 0.05$.

RESULT AND ANALYSIS : The result of this study showed that preschool knowledge had significance level of p= 0.001 in treatment group, while in control group was p=0.157, for preschool attitude p=0.001, while in control group was p=0.317, for preschool skin hygiene behavior p=0.001, while in control group was p=1.000, for preschool mouth hygiene behavior p=0.001, while in control group was p=1.000, for preschool hand hygiene behavior p= 0.001, while in control group was p=0.317, and for preschool hair hygiene behavior p=0.000, while in control group was p=0.317. The result of Mann Whitney U test was p=0.000, it means there was different in knowledge, attitude and personal hygiene behavior between treatment and control groups.

DISCUSSION : It can be concluded that there is significant effect of health education by playing puzzle to improve preschool's personal hygiene behavior. Puzzle is an alternative education media with innovative methods, so it can improve coginitive ability and be exercise to solve their own problem.

Key words : Health Education, Personal Hygiene, Preschool, Puzzle

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A-2 (07-1) Table Clinic Activity in Teaching Preventive Dentistry in Third Year KKU Dental Students: a Case Study

A. Rattanathongkom[1], J. Paphangkorakit[1], S. Luengpailin[1], T. Damrongrungruang[1], N. Poomat[1], T. Plangkoon jorns[1], R. Noisombut[1], P. Pisek[1], P. Phajongviriyatorn[1], P. Thanyakob[1] and S. Rattanathongkom[2]

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BACKGROUND : Preventive Dentistry Practice is a course for teaching third year dental students. These are a lot of difficult and complicated contents for students to understand. Therefore, a table clinic activity (TCA), brief oral presentation in areas of clinical application and techniques, was applied for teaching.

OBJECTIVE : To evaluate students learning activities, opinion and attitude toward TCA oriented teaching.

METHOD : Seventy third year students, enrolling in this course in second semester 2014, were assigned to actively participate in this process. The following learning outcomes and activities were evaluated by: 1) student behavior and classroom activities, 2) individual staff and student interview, 3) student report and presentation 4) questionnaire. After 10 lectures session in basic knowledge given by instructors, all students were divided into 7 groups, one patient was assigned for each group. All patients had different oral health problems. Each group of student followed patient in 4 sessions for: 1) assessment patient's problems, 2) obtaining an appropriate preventive planning programs, 3) applying the preventive treatment to the patients, 4) re-assessment the patients. One morning-session (TCA) was organized to allow each group of the students to present their results. All students and teachers discussed and shared knowledge for each patient case.

RESULTS : The most students (94.2%) actively participated in learning and were able to understand the whole contents; they (95.65%) were able to integrate the concept of holistic approach into the real patients. During interviews session, 28 students showed good attitudes and gain experiences toward the learning process, 16 students felt proud to present their cases and shared the experience closely with their teachers.

CONCLUSION : TCA can possibly promote the integration of holistic approach in practical preventive dentistry for students, who have not yet met the real patients. Students can learn cooperatively, and share their opinions together and closely with their teachers.

A-3 (07-2) Accuracy of Intraoral Surveyor Compared to Ney Surveyor on Customized Models

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Faculty of Dentistry, Khon Kaen University, Thailand

Preparation of abutment teeth paralleling to the path of insertion is crucial in the production of removable or fixed partial denture. This step of prosthesis fabrication is generally difficult for dental students and the use of aiding device such as dental surveyor can be helpful. We developed a prototype of intraoral surveyor to assist the students' practice in preparation of abutment teeth and determination of optimum guiding planes on a mannequin in dental simulation laboratory. The purpose of this study was to determine the accuracy of the intraoral surveyor compared to standard Ney surveyor. Survey lines were produced on 12 customized models using two different surveyors. The investigator who performed the surveys was a lecturer in the Department of Prosthodontics who had more than 15 years of experience in the field. The differences in the survey lines created by the two surveyors on each sample were measured by a stereomicroscope. The difference of ≤0.2 mm was considered as acceptable. The Westlake's test of equivalence demonstrated that the mean distance between the survey lines of the intraoral surveyor and the Ney surveyor was significantly greater than 0.2 mm (mean = 0.35 mm, 95% confidence interval = 0.24-0.47, $p < 0.001$). The results suggest that this prototype of the intraoral surveyor was not equivalent to the Ney surveyor. Further development is therefore needed to improve the accuracy of this intraoral surveyor for use in aiding dental students' practice.

A-4 (18-1) Comparative Study on Accuracy of Three Electronic Apex Locators Before and After Extraction of Lower 1st Permanent Molars

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The aim of this study was to compare the accuracy of three electronic apex locators (EALs) in determining working tooth length. Working length of each tooth was carried out before and after the tooth was extracted, and thus the measurements in vivo were compared with the real tooth length (RTL) measured in vitro. Thirty lower 1st permanent molars, scheduled for extraction, were selected for the study. After obtaining the consent from the patients, a preoperative radiograph was taken, the access opening was prepared, and the occlusal reference plane was maked in each tooth. Working length was determined by using three apex locators; Sybro Endo mini apex locator, Foramatron-D 10 apex locator and C Root apex locator. For all the electronic measurement of canal length, #15 K-file was used. During the measurement, the canal was irrigated with 2.5% sodium hypochlorite. Each tooth was then extracted and the real tooth length was measured by inserting #15 K file into the root canal until the file tip was just visible at the level of the apical foramen. This procedure was carried out under a magnifying glass at a magnification of 5x. All the measured lengths were the distance from the coronal reference plane to the apical foramen. The results obtained from in vivo measurement were compared with the corresponding RTL. In locating the apical foramen, the accuracy of Foramatron D10 apex locator was 97.8%, of C Root apex locator was 95.6%, and of SybroEndo mini apex locator was 94.5%. The one-way ANOVA test showed no significant difference in accuracy of all types of EALs devices in mesiobuccal, mesiolingual and distal canals ($p=0.987$ in mesiobuccal canal, $p=0.997$ in mesiolingual & $p=0.952$ in distal canal of lower 1st permanent molars). It is concluded that the electronic apex locators tested in this study exhibited clinically acceptable accuracy within the range of ±0.5 mm.

A-5 (21-1) Metric and Non-metric Characteristics in the Dentition of Children/adolescents with Down Syndrome Attending an Institution in Jaffna, Northern Sri Lanka

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BACKGROUND : Abnormal growth in Down syndrome is reflected by variable reduction in size and simplification in form of many physical traits.
OBJECTIVES : This study aimed to investigate the metric and non-metric dental characteristics of a group of Down syndrome individuals in northern Sri Lanka.
MATERIALS AND METHOD : Twenty six dental casts collected from children/adolescents with Down syndrome were used. Buccolingual and mesiodistal crown diameters of teeth were measured using digital venire calliper to the nearest 0.01mm. Fifteen non-metric features were recorded using the Arizona State University Dental Anthropology System and frequencies of occurrence were calculated. Sexual dimorphism of metric data and fluctuating bilateral asymmetry of metric and non-metric features were also analysed.
RESULTS : Teeth were small in Down syndrome individuals. Tooth dimensions were generally greater in male than female. However, statistically significant differences were observed only in mandibular central incisor, and mandibular first and second molars. Fluctuating asymmetry was high in metric dimensions. They also showed higher prevalence of winging (16%), shovelling (30%) and double shovelling (15%) in maxillary central incisors than those in Sri Lankan people. They showed a high prevalence of cusp 5 (90.2%) and cusp of Carabelli (80.9%) in maxillary first molars, multiple lingual cusps (89.4%) in mandibular second premolars, hypocone (83.3%) in maxillary second molar and anterior fovea (70%) in mandibular first molar. Deflecting wrinkle in mandibular first molar showed the lowest prevalence (5%). Down syndrome individuals showed high bilateral asymmetry in Y-shaped groove pattern, and in cusp 6 in mandibular first molars.
CONCLUSION : Down syndrome individuals show microdontia, and minimal sexual dimorphism. Non-metric traits of them show both the features of indodont and sinodont dental pattern. They show high fluctuation bilateral asymmetry in both metric and non-metric characteristics. These results are consistent with the concept of amplifies developmental disturbances and instability for dental traits in Down syndrome.

A-6 (23-1) An Assessment of the Class "International Future Dentistry with the Outcome of Researches 2014 and 2015" with a Video Conference System

H. Oka[1], M.D. Ariani[1], P.T.T. Nguyen[1] and K. Kato[1,2]

[1] Department of International Collaboration Development for Dentistry (ICDD), Institute of Biomedical & Health Sciences, Hiroshima University, Japan, [2] Department of Biomaterials, Institute of Biomedical & Health Sciences, Hiroshima University, Japan

BACKGROUND : Information and Communication Technology has developed and we can apply this technology to our education. Since 2012, Hiroshima University Faculty of Dentistry (HUD) has shared some lectures in dental schools with our oversea sister schools by video conference systems. Since 2014, we have established the class "International Future Dentistry with the Outcome of Researches," and shared the lectures with Airlangga University in Indonesia, University of Medicine and Pharmacy at Ho Chi Minh City in Vietnam and University of Sheffield in the United Kingdom. The class objectives are; being able to (1) understand the topics in dentistry with global view point, (2) understand the relationship between clinic and the research outcomes, (3) explain how to apply the result of researches, (4) develop international dental education for undergraduate dental students. The lectures in the class conducted in English and have the topics related to trend of biology and genome medicine, and practice evidence-based and patient-oriented dentistry. Each lecture includes contents about the relationship between clinical treatment and the research outcomes.

METHODS : To assess the new class and the facilities, we did a questionnaire survey to the HUD students in the class at the end of the class 2015.

RESULTS : 30% of HUD students answered they could receive many opinions/ideas from different viewpoints for one topics in this class. About the facilities, 60% of the HUD students didn't have big problems to follow the slides and to catch the sounds through the video conference systems. However, 50% of them answered that they could not feel the lecturers' attention to them through video conference system clearly.

CONCLUSION : To share classes among several schools is meaningful not only for receiving lectures from speakers in abroad but also for understanding different viewpoints through questions and discussions. We need to develop appropriate conditions to share live lectures more effectively from abroad.

A-7 (23-2) What Do You Think? —A Questionnaire Survey to Dental Students in UMPH and HU by Participants of Short-term Visit 10 Days Program 2015—

M. Kato[1], K. Okamoto[1], H. Nagao[1] and H. Oka[2]

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BACKGROUND : Hiroshima University (HU) Faculty of Dentistry has newly established the International Dental Course program since 2011. This is the program that students from oversea partner universities study dental subjects in Hiroshima for four years. In addition, "Short-term Visit (SV) 10 days programs" has been conducted during the same periods. SV 10 days programs are exchange programs which HU dental students visit partner universities for 10 days. The aims of those programs are not only the friendship between the institutions becomes closer but also students can understand the importance of mutual understanding, experience harmonious coexistence and develop each other. In 2014, three HU dental students visited University of Medicine and Pharmacy at Ho Chi Minh City (UMPH).

STUDY OBJECTIVE : The objective of this study is to understand the general views and their backgrounds of dental students in UMPH and HU.

DESIGN AND METHODS : We did a questionnaire survey with 10 questions to dental students in UMPH and HU. The questions included their English skills and dental problems in the country, features of the university, future career plans.

RESULTS : 174 UMPH students (the 4-6th grade) and 35 HU students (the 5th grade) replied. As dental problems in the country, UMPH students answered "high prevalence of dental caries." On the other hands, HU students payed attention to the influence of the super aging society. UMPH students recognized that they frequently used English "reading" skills and HU students used English "listening and reading" skills.

CONCLUSIONS : Through SV program 2015, we noticed cultural and national differences between UMPH and HU. Features of university and future career plans might be related to education system of each university. By conducting this questionnaire survey to many dental students both universities, we could know general views and backgrounds more widely.

A-8 (23-4) The Current Status of Instruction and Curricular Content in Forensic Odontology in Asian Countries I. Results from the Pilot Questionnaire Survey

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BACKGROUND : Dentists play an important role locally and nationally in assisting in the identification of the victim of the mass fatality incidents. Moreover, incidences can involve people of different nationalities in this globalization era. Therefore, it is very important to share the common knowledge and skills about forensic odontology among dentists in the world and same region for the collaboration. This is the first survey, which was conducted to assess the current status and curricular content of instruction in forensic odontology education in dental schools of Asian countries.

METHODS : The questionnaire was sent to twelve dental schools.

RESULTS : Ten schools from Cambodia, Indonesia, Malaysia, Philippine, Taiwan, Thailand, Vietnam and Japan replied. Our results indicate 20% of dental schools who responded to the survey still did not offer any formal education in forensic odontology. The other 80% of those had some dental association/societies/institutions in the country working for forensic issue and they also had the contents related to "Individual identification" in DDS or BDS curriculum regardless to the exist of a subject "forensic odontology". Three schools answered that they have departments related to forensic odontology in their hospital or college. In addition, seven schools from five countries answered that they knew some trained forensic odontologists in their countries.

CONCLUSION : Although some forensic dental faculties are trained to assist law enforcement agencies, it seems to be there are differences in the depth of the forensic dental education among Asian dental schools. Based on the results of this survey, a series of recommendation for structuring the forensic science curriculum in dental schools is necessary. In the case of Japan, there is the Model Core Curriculum for dental education. However, although some schools have a subject, "Forensic Odontology", others only have special lectures about topics related to that. Therefore, we suggest that formal instruction in the principles of forensic odontology should be a goal of organized dentistry in Asia.

A-9 (23-5) How and What We Learned? —HUD Short-term Visit 10days Program 2015—

M. Kawamoto, S. Tsuyama, Y. Kimura, N. Yamakado, T. Akitomo, H. Nakamura, J. Cho, M. Tanaka, K. Tsutiya, M. Kato, K. Okamoto, A. Fujii, M. Kiyama, H. Nagao, R. Hiura and T. Kobayashi

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BACKGROUND : Short-term Visit (SV) 10days Programs of Hiroshima University Faculty of Dentistry (HUD) is a global personnel training program by collaborations with HUD sister schools. At HUD, we have learnt the dental subjects with a Japanese-English dual linguistic education system; all the lectures given in these two languages because students need to understand special Japanese term for learning about Japanese system. SV 10days Programs link to the education system and we can develop dental networks with multicultural dental and social experiences in abroad. In this presentation, we would like to report the SV 10days Program 2015 and show the splendor of the studying abroad.

SUMMARY OF WORK : We separately joined the program at Taipei Medical University in Taiwan (3 students), University of Sheffield in England (3 students), University of British Columbia in Canada (4 students), University of Medicine or Pharmacy Ho Chi Minh City in Vietnam (3 students), Khon Kaen University in Thailand (3 students). We participated in this program with various aims and purposes; to improve the ability of English conversation, to visit the dentistry spot like dental clinic or hospital, to experience foreign cultures, to consider about the future life and so on.

SUMMARY OF RESULTS : We learned about culture, tradition and of course dentistry of each country. We were able to exchange many opinions by meeting various professors, teachers and students at each university.

CONCLUSION : It's very important to share the information and develop networking among the future professionals. The SV program gave us a big opportunity to think about the future concretely.

B-1 (01-2) Modulation Mechanism of Immune Respons From Non Atopy into Atopy Rat After *Porphyromonas gingivalis* Lipopolysacaride Exposure

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BACKGROUND AND RATIONALE : In pediatric dentistry, gingivitis is a common disease, where one of the pathogens causing the problem is *Porphyromonas gingivalis* (Pg). It is indicated in some research that Pg has a significant role in activating cytokines which are responsible for allergies. Allergic asthma is a respiratory disease that often affects the various age, but the prevalence is higher in children. During these allergic asthma is still considered as a genetic disease and it is believed that the pathogenesis of asthma occurs only in atopic individuals. However, some previous studies found that asthma can be found in subjects who previously had no atopy history.

STUDY OBJECTIVE is an attempt to explain the mechanism of pathogenesis of asthma in non-atopic subjects due to exposure to the bacteria Pg and their products. Porphyromonas gingivalis which has a form of endotoxin lipopolysaccharide has a role to activate TLR2 and change Th0 to Th2 direction, so previously non-atopic subjects become atopy. *Porphyromonas gingivalis* bacteria can be passed among individuals, hence the LPS in it was also moved. With a certain dose of non atopic, it is proved that individuals who are exposed to LPS into atopy can develop into asthma.

METHODS : Wistar rats were used in this study, which represents non-atopic subjects as experimental animals, given the exposure to LPS Pg then four days later examined levels of Th1, Th2, and Elisa were used in this study.

RESULT : There is a shifting from a non-atopy rat became an atopy rat. This is evidenced by increasing Th2 in non atopy rat cause of LPS Pg exposure.

CONCLUSION : Clinicians can reduce the prevalence of asthma by controlling amount of Pg bacteria.

Key words : LPS, *Porphyromonas gingivalis*, Asthma.

B-2 (01-3) Cellular Changes in the Mucosal Tissue Incision Wound of Wistar Rats After Administered with 40% Topical Gel Bovine Colostrum

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BACKGROUND : Povidone iodine as topical administration may be toxic and has a bad taste for intra oral lesion in children. Thus, alternatives of topical administration is essential to shorten the wound healing proses while reducing discomfort that arise in children.

PURPOSE : The objective of this research was to determine the number of inflammatory cells, fibroblast, and new blood vessel on rat mucosa wound, after previously administered with topical administration of 40% pure bovine colostrum gel.

METHODS : A total sample of 9 strains of male Wistar rats (Rattusnorvegicus L) were divided into 3 groups. The first group was controlled group, rat mucosa were incised followed by irrigation with sterile distilled water. The second group, incision followed by irrigation with povidone iodine 10%. The third group, incision followed by irrigation with 40% pure bovine colostrum gel. Irrigation was conducted twice a day on all rats. Wound tissues biopsy were performed at 48 hours after incision. Neutrophils (polymorphonuclear), lymphocytes, fibroblast, and new blood vessels were counted under microscope. Data were tested using One way Anova andd Tukey HSD with significance level of 95%.

RESULT : The number of lymphocytes, fibroblast, and new blood vessels were higher on 40% pure bovine colostrum gel administration than that in the other groups. The number of neutrophils in the group with 40% pure bovine colostrum gel was lower compared to the other groups.

CONCLUSION : This study showed that 40% pure bovine colostrum gel administration increased lymphocytes, fibroblast, and new blood vessels number and lowering neutrophils, which might be indicators of wound healing.

Key words : Bovine colostrum, wound healing, polymorphonuclear (PMN), lymphocytes, fibroblast, new blood vessel

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B-3 (04-1) Basic Fibroblast Growth Factor Induced Interleukin 6 Expression by Stem Cell Isolated from Human Exfoliated Deciduous Teeth

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BACKGROUND : Basic fibroblast growth factor (bFGF) and interleukin-6 (IL-6) regulate pluripotent stem cells expression, particularly *REX-1*, in stem cells isolated from human exfoliated deciduous teeth (SHEDs). Though, their interaction was not yet unknown.

OBJECTIVE : The aim of present study was to investigate the effect of bFGF on IL-6 expression in SHEDs.

METHODS : Cells were isolated from dental pulp tissues of human deciduous teeth. Flow cytometry was used to determined mesenchymal stem cell marker expression and the osteogenic and adipogenic differentiation potential was also determined. IL-6 mRNA and protein expression was determined using real-time quantitative polymerase chain reaction and enzyme linked immunosorbent assay, respectively.

RESULTS : Cells expressed CD44, CD73, CD90, CD105 but not CD45. Further, the upregulation of osteogenic and adipogenic marker genes was observed upon maintaining cells in osteogenic and adipogenic inductive medium, respectively. bFGF induced IL-6 mRNA and protein expression in dose dependent manner. The significant upregulation of IL-6 expression was noted at the bFGF concentration of 10 ng/mL. The intracellular mechanism of bFGF-induced IL-6 expression was also determined.

CONCLUSION : bFGF enhanced IL-6 expression by SHEDs and this mechanism may participate in bFGF regulating stemness maintenance.

B-4 (04-2) Chemogenomic Analysis of Antifungal Mechanisms of Chitosan

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BACKGROUND AND RATIONALE : Oral candidiasis is a common opportunistic infection in immunocompromised patients. With increasing prevalence of antifungal drug resistance, alternative/adjunctive antifungal agents are in demand.

OBJECTIVE : To use a chemogenomic approach in *Saccharomyces cerevisiae* model to investigate the antifungal mechanisms of chitosan, a natural product with high biocompatibility and antimicrobial activity.

MATERIALS AND METHODS : Broth dilution and agar dilution assays were employed to determine the Minimum Fungicidal Concentration (MFC) and sublethal concentration, respectively, of a water-soluble chitosan (molecular weight 150-200 kD) against *S. cerevisiae*. The sublethal concentration was used to screen the haploid yeast deletion library, a collection of yeast strains with single deletions of virtually all open reading frames in the yeast genome. Deletion strains that showed significantly less growth than the wild type were selected and rescreened. The list of genes whose deletion led to hypersensitive to chitosan was analyzed using Gene ontology (GO) analysis tools on *Saccharomyces* genome database. To determine the effect of chitosan on susceptibility of common oral *Candida* species to amphotericin B and fluconazole, e-test strips was used to determine the minimum inhibitory concentration (MIC) of the drugs in the presence and absence of chitosan in the media.

RESULTS : We identified yeast deletion strains with hypersensitivity to the hight molecular weight water-soluble chitosan. The group was enriched for genes involved in membrane biosynthesis and endosomal sorting complexes. This result suggested that the antifungal activity might be enhanced when we combine chitosan with antifungal drugs that target the yeast cell membrane. This hypothesis was explored in several *Candida* species. Interestingly, the MICs of fluconazole and amphotericin B for certain *Candida* species were decreased by 1.5-4 folds in the presence of chitosan.

CONCLUSION : Water-soluble chitosan likely targets the fungal cell membrane and could enhance the activity of amphotericin B and fluconazole.

Key words : Antifungal agent, *Candida*, chitosan

B-5 (05-1) Rugoscopy among the Malaysian Chinese Population: A Pedigree Analysis

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BACKGROUND : Rugoscopy is the study of palatal rugae. It is found to be unique to individuals and can be used for personal identification which is important for any forensic investigation. However, palatal rugae for pedigree analysis is yet to be explored.

OBJECTIVES : To determine the relationship and similarities between palatal rugae type among female students and their parents. The objective is to identify the majority rugae type among the Malaysian Chinese population.

EXPERIMENTAL METHOD : The sample size constituted of 23 families accounting for a total of 69 individuals (23 fathers, 23 mothers and 23 daughters). Palatal rugae were analysed using the dental casts. The parameters recorded were length, shape, unification, direction, site of rugae and total number. Mann-Whitney U test was used to test the relationship between both parents and child.

RESULTS : It was found that the father's palatal rugae type similar to his child in terms of primary length, curved, wavy, circular, total of unification, diverging, converging, forwardly and total number on the right side. The mother's palatal rugae type is similar to her child's in terms of primary, curved, wavy, straight, circular, total of unification, diverging, converging, forwardly, left, right and total of rugae. It was found that primary length, straight shape, diverging unification, forwardly directed pattern and total number of rugae found on the left side of the palate is predominant for Malaysian Chinese population.

CONCLUSION : Pedigree analysis can be done by determining the similarities of those parameters of palatal rugae and personal identification is possible as it is unique to every individual. These features can definitely aid as a supplemental tool for forensic identification.

Key words : Rugoscopy, Palatal Rugae, Pedigree Analysis, Malaysian Chinese

B-6 (07-3) Expression of p73 Isoforms and Aurora Kinases in Oral Squamous Cell Carcinoma Cell Lines

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BACKGROUND : As the expression levels of Aurora kinases, particularly Aurora-A and Aurora-B, are up-regulated in a broad range of human cancer cell lines and tissues including oral cancer, many studies have thus considered them as potential prognostic markers and targets for cancer treatment. Furthermore, some studies have demonstrated the interaction between p53 family members and Aurora kinases. However, differential expression profiles of Aurora kinases and p73 isoforms in oral cancer cell lines are not fully investigated.

OBJECTIVE : To investigate the expression of Aurora kinase family members including Aurora-A and Aurora-B as well as p73 isoforms in *p53*-mutated oral squamous cell carcinoma (OSCC) cell lines, ORL-48T and ORL-136T as compared to the normal human gingival epithelial cells.

EXPERIMENTAL METHODS : The expression levels of Aurora-A, Aurora-B, and p73 isoforms in *p53*-mutated OSCC cell lines, ORL-48T and ORL-136T as well as the normal human gingival epithelial cells were examined by real-time reverse transcription PCR and Western blot analysis.

RESULTS : ORL-136T, but not ORL-48T, expressed a significant increase in mRNA and protein levels of Aurora-A as compared to the normal gingival epithelial cells. Although ORL-136T did not demonstrate a significantly increased level of Aurora-B mRNA, this cell line demonstrated a remarkable higher level of Aurora-B protein than the normal gingival epithelial cells. In addition, ORL-48T and ORL-136T expressed higher mRNA levels of p73 isoforms, including TAp73, ΔEx2p73, and ΔEx2/3p73, than the normal gingival epithelial cells. Both oral cancer cell lines expressed TAp73 and ΔNp73 protein and they expressed higher level of ΔNp73 protein than the normal gingival epithelial cells.

CONCLUSION : Aurora kinases and p73 isoforms are aberrantly expressed in these two oral cancer cell lines. These data support an essential role of Aurora kinases and p73 isoforms in tumourigenesis of oral cancer (MRG5680015 to PK).

B-7 (07-4) Anti-fungal Effectiveness of Dental Stone Incorporated with Lodopropynyl Butylcarbamate

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BACKGROUND : The dental stone is widely used in dentistry for study and master model. However, when dental stone be exposed to moisture or contaminated from remained food in the saliva, owing to the impression making, the fungi will grow and result in the corrosion of the model surface. If the fungus left out to proliferate and produce numerous spores that can spread in the air will harm to the health of anyone who inhale or contact with the spores.

OBJECTIVE : This research studied in the effectiveness of growth inhibition of fungus in dental stone mixed with Iodopropynyl butylcarbamate (IPBC), an antifungal agent. The objective is to compare the effectiveness of growth inhibition of *Aspergillus fumigatus* in dental stone incorporated with IPBC to the conventional.

METHOD : The modified IPBC dental stones at concentration of 100 1,000 and 10,000 ppm by weight were tested with the zone of fungal growth inhibition by Agar well diffusion assay and chose the least concentration that effected to be tested with the period of resisting.

RESULT : The modified IPBC dental stones at concentration of 100 1,000 and 10,000 ppm have the average diameters of the zone of fungal growth inhibition were 12.73±0.06 21.43±0.35 and 28.22±0.14 millimeters, respectively. In the test of period of resisting, the concentration of 100 ppm was chosen and found that it can inhibit the fungal growth during all the time for 6 weeks of test.

CONCLUSION : The dental stones incorporated with IPBC at concentration range of 100 to 10,000 ppm can inhibit the fungal growth. The increasing of the zone of inhibition will be raised according to the concentration level of IPBC. In practically, we can use the dental stone incorporated with IPBC at concentration of 100 ppm at least 6 weeks long, safely, without the growth of *Aspergillus fumigatus*.

B-8 (07-5) Prevalence of Abnormalities of Teeth and Jaws on Panoramic Radiographs in Thalassaemic Patients

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INTRODUCTION : Thalassaemia is a form of inherited autosomal recessive blood disorder characterized by abnormal formation of hemoglobin and considered the most common genetic disorder world-wide. Previous literature reviewed a little information about the panoramic radiographic changes of teeth and jaws in thalassaemia.

OBJECTIVES : The purpose of the present study, therefore, was to evaluate the abnormalities of teeth and jaws on panoramic radiographs in thalassaemic patients.

MATERIAL AND METHODS : 163 patients (male=72 (44.2%), female=91 (55.8%); aged 1.9-28.6 year-old (mean±SD; 12.4±5.8 year-old) were studied. Only 148 (90.8%) patients, however, were taken the panoramic radiographs. The radiographs were reviewed the occurrence of any of the following radiographic sign: 1. Root lengths and crown-body ratio, 2. Supernumerary teeth, 3. Taurodontism, 4. Germination, 5. Dental age estimation, 6. Small maxillary sinuses, 7. Identification of the inferior alveolar canal, and 8. Mandibular cortical erosion. All data were presented in descriptive data. The relationship between thalassaemia grouped by genetics and abnormalities of teeth and jaws on panoramic radiographs were evaluated by logistic regression analysis.

RESULTS : The prevalence of abnormalities of teeth, small maxillary sinuses, absence of inferior alveolar canal and mandibular cortical erosion were 68.2%, 52.7%, 50.7%, and 29.7%, respectively. Root lengths and crown-body ratio were 2.25±0.41 and 2.38±0.39 mm in male and female, respectively. The chronological and dental age differences were 0.23±1.08 and 0.72±1.07 in male and female, respectively. Alpha-thalassaemia group was found abnormalities of teeth more than Beta-thalassaemia group 3.3 times. (OR=3.3, 95% CI=1.2-9.5; p=0.02). Furthermore, Alpha and Beta Thalassemia group was found small maxillary sinuses less than Beta-thalassaemia group 0.1 times (OR=0.1, 95% CI=0-0.4; p<0.001).

CONCLUSION : The thalassaemic patients may be associated with abnormalities of teeth and small maxillary sinuses on panoramic radiographs.

Key words : thalassaemia, panoramic radiograph, jaw abnormalities, tooth

B-9 (09-1) Using Transgenic Mouse Models as an Approach to Study Potential Stem Cell Subpopulation in Salivary Gland

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BACKGROUND : Salivary gland damage decreases saliva secretion, resulting in difficulties in mastication and oral diseases, thereby patient's poor quality of life. Tissue engineering is a promising approach for salivary gland regeneration but its success is still limited by an effective source of stem/progenitor cells, inducers, and scaffolds. Salivary gland mesenchymal cells were distinguished from epithelium by a neural crest marker, platelet derived growth factor receptor-alpha (PDGFRa), and previously shown to support salivary gland differentiation. Nevertheless, a stem/progenitor cell function of salivary gland mesenchymal cells is still undetermined.

OBJECTIVES : To investigate if PDGFRa+ subpopulation of neural crest-derived cells, in addition to a role as supportive cells, may function as stem/progenitor cells in salivary gland regeneration.

EXPERIMENTAL METHODS : PDGFRa transgenic mice were used to localize PDGFRa expressing mesenchymal cells in submandibular salivary glands. These PDGFRa+ cells were isolated, expanded, characterized, and examined their *in vitro* differentiation capacity. PDGFRa transgenic mice were irradiated for the whole body with the low dose and then observed for one month post-operation to determine a derivative of mesenchymal cells to acinar tissues by the genetic lineage tracing.

RESULTS : Histology of submandibular salivary glands demonstrated that PDGFRa expressing cells were located in mesenchymal stromal tissues, indicating that PDGFRa was able to use as a mesenchymal marker distinguished from acinar epithelial cells. We successfully isolated and expanded PDGFRa+ cells from submandibular salivary glands in our culture condition and showed that these cells represented spindle-shaped mesenchymal cells. These isolated cells also expressed neural crest (PDGFRa and SOX10) and mesenchymal (COL1 and SMA) markers, but not epithelial marker (E-cad). This indicated that our culture maintained a majority of neural crest-derived salivary gland mesenchymal cells. *In vitro* 3D differentiation on matrigel (MG), cells on MG formed acinar-like structures whereas undifferentiated cells on tissue culture polystyrene (TCPS) formed only a confluent monolayer. In addition, these acinar-like structures on MG, but not on TCPS were positively stained for amylase-1, an enzymatic marker in salivary glands. Q-RT-PCR also showed higher level of specific salivary gland gene markers (*Amy-1*, *Aqp-5*, and *ZO-1*) in that on MG, compared to TCPS, confirming a differentiation capacity of PDGFRa expressing cells. Interestingly, irradiated murine submandibular, sublingual, and parotid salivary glands in PDGFRa transgenic mice showed that some acinar tissues were derived from PDGFRa expressing mesenchymal cells. Those acinar tissues also showed positive staining for amylase-1 and aquaporin-5, suggesting that they are functional. This genetic tracing data confirmed our *in vitro* differentiation's result and indicated the potential stem/progenitor cell function of salivary gland mesenchymal cells.

CONCLUSION : We accomplished to isolate, and expand PDGFRa expressing salivary gland mesenchymal cells. The *in vitro* and *in vivo* studies suggest that PDGFRa cell subpopulation functions as stem/progenitor cells in the salivary gland. This result would give us a great hope to use this cell subpopulation as a stem/progenitor cell source in salivary gland regeneration and tissue engineering.

B-10 (09-2) Inhibitory Effect of *Moringa oleifera* L. Seed Oil on Oral Pathogens

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BACKGROUND : *Moringa oleifera* L. or drumstick tree is a small deciduous tree found in many tropic and subtropics regions worldwide. It is used as a highly nutritive vegetable in many countries. Various parts of *M. oleifera* tree such as root, root bark, leaves, flowers, and unripe pods are commonly consumed and they are having some medicinal properties.

OBJECTIVE : Essential oil extracted from *M. oleifera* seeds was assayed for the evaluation of antimicrobial activities against oral pathogens.

EXPERIMENTAL METHODS : Essential oil extracted from *M. oleifera* seed was obtained from Tea oil and Plant Oils Development Center, Thailand. The stock solution of the oil was prepared in 10% dimethyl sulfoxide (DMSO) and further diluted in distilled water to give the final concentration of 50% (v/v) for the use in the experiment. Oral pathogens used for the evaluation were *Streptococcus mutans* KPSK2, *Lactobacillus casei* ATCC 6363, *Candida albicans* ATCC 10231, *Porphyromonas gingivalis* W50, and *Actinomyces israelii* ATCC 10048.

The microbial growth inhibitory potential of the oil was initially determined using agar disk diffusion method. Then the minimum inhibitory concentration (MIC) values were also evaluated using agar dilution method. A 0.2% chlorhexidine gluconate and 5% DMSO solution served as positive control and negative control, respectively.

RESULTS : *M. oleifera* oil had antimicrobial activity against *C. albicans*, *P. gingivalis* and *A. israelii* with the zones of inhibition ranged from 10 to 13 mm. MIC values for *C. albicans*, *P. gingivalis* and *A. israelii* were 3.12, 0.32 and 0.39 % v/v, respectively. No inhibitory effects were observed on *S. mutans* and *L. casei*.

CONCLUSION : Essential oil extracted from *M. oleifera* seeds can inhibit the *in vitro* growth of some oral pathogens. It appears to be a promising source of new alternative antimicrobial agent that may be used for the prevention or treatment of some oral infections.

B-11 (13-1) Expression and Function of Homeobox Gene Lhx8 during Tooth Development

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Lhx8 is a transcription factor of the LIM-homeobox gene family, and plays pivotal roles in the morphogenesis and cell fate specification of multiple organs. Previous study using Lhx8 and Lhx6 double knockout mice revealed that deletion of Lhx6/8 interrupts tooth initiation and dental mesenchymal specification, with the phenotype of missing molars. However, Lhx8 functions and signaling pathways are largely elusive.

In this study, we sought to probe Lhx8 expression in the embryonic and postnatal tooth organ; explore Lhx8 function in tooth development, odontoblast differentiation and dentinogenesis by silencing and overexpressing Lhx8 in different models; clarify the transcriptome change in Lhx8 overexpressed human dental pulp stem/progenitor cells (hDPSCs) and investigate the related signal pathways essential for the function of Lhx8. Our study here provides essential information for understanding the role of Lhx8 in tooth development and thus tooth regeneration.

In the process of tooth development, Lhx8 was found to be intensively and exclusively expressed in neural crest derived ectomesenchyme and dental mesenchyme, continuing to restrict to dental papilla and odontoblast and gradually decrease over time. Lhx8 knockdown leads to accelerated tooth development and dentinogenesis, whereas Lhx8 overexpression delays/inhibits the process, suggesting that Lhx8 negatively regulate odontoblast differentiation and dentinogenesis. Excessive Lhx8 negatively regulates mineralization, odontoblast differentiation and dentin maturation, whereas exerts minor effects on cell proliferation and migration. Lhx8 activates Wnt and TGFβ signaling pathways, which contribute importantly in Lhx8 mediated fine-tuning of tooth development. During early tooth development, high-level Lhx8 specifies dental mesenchyme by activating both pathways, whereas in later differentiation stage, the waning of Lhx8 decreases the activation of Wnt and TGFβ signaling pathway, guaranteeing odontoblast differentiation and tooth development.

Together, Lhx8 plays an important role in regulation of tooth development and regeneration by fine-turning Wnt and TGFb signaling.

B-12 (23-6) Differentiation of Induced Pluripotent Stem Cells into Dental Epithelial-like Cells in a Defined Culture Condition

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Tooth regeneration is one of the most significant challenges in regenerative dentistry. Recent studies have successfully generated tooth-like structure by mimicking the reciprocal interaction between dental epithelial and mesenchymal cells in tooth organogenesis. However, clinical applications of such technologies for tooth regeneration are limited primarily due to the lack of appropriate source of dental epithelial cells. Induced pluripotent stem cells (iPS) are attractive as an alternative source for dental epithelial cells due to their unique characteristics. Although there were several reports that dealt with iPS cells as the source of dental epithelial cells, however, an efficient procedure for dental epithelial differentiation remains to be established for the future applications in regenerative dentistry. In the present study, we examined the effect of neurotrophin-4 (NT-4) on the differentiation of iPS cells into dental epithelial cells. Addition of NT-4 during the formation of embryoid body (EB) triggered up-regulation of epithelial markers (p63 and CK14) significantly, suggesting that NT-4 provides an inductive microenvironment for the differentiation of iPS cells into dental epithelial cells. Expansion under serum-free culture conditions improve the formation of cells with cobblestone-like morphology and significantly down-regulated the expression of Oct4 and K18. Phenotypic analysis revealed high expression of a dental epithelial surface marker, CD49f, on these cells. Formation of iPS-derived dental epithelial-like cells was further confirmed by the high expressions of ameloblast-specific markers (AMBN, AMG, DSPP and DMP-1). These results suggest that the addition of NT-4 during the formation of EB together with the serum-free culture condition promoted the differentiation of iPS cells into dental epithelial-like cells.

Key words : Induced pluripotent stem cells, dental-epithelial-like cells, neurotophin-4

B-13 (23-7) Distinction between Cell Proliferation and Apoptosis Signals Regulated by BDNF in Human Periodontal Ligament Cells and Gingival Epithelial Cells

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BACKGROUND : Brain-derived neurotrophic factor (BDNF) is known to play a role in proliferation and differentiation in various types of cells. Previously, we have reported that BDNF enhances periodontal tissue regeneration in dog periodontal defect model. Importantly, the down growth of gingival epithelium which comprises a major obstacle to the regeneration was not observed. For the clinical application of BDNF, it is indispensable to reveal the molecular mechanism underlying this key finding. On the other hands, it is reported that BDNF induces neuronal cells apoptosis via p75-JNK signaling.

OBJECTIVE : The aim of this study is to test whether BDNF facilitates apoptosis via p75-JNK cascade of human gingival epithelial cells in comparison with human periodontal ligament cells.

EXPERIMENTAL METHODS : Immortalized human gingival epithelial cells (OBA9) and immortalized human periodontal ligament cells (HPL cells) were employed. The cells were exposed to BDNF. The cell proliferation and apoptosis was determined by BrdU assay and TUNEL staining, respectively. In addition, phosphorylation of ERK, JNK and cleaved-caspase3 were detected by immunoblotting. Furthermore, an inhibition assay was conducted by using JNK inhibitor, SP600125 or p75 siRNA transfection.

RESULTS : BDNF treatment facilitated cell proliferation and phosphorylation of ERK in HPL cells. However, contrary to HPL cells, OBA9 showed neither cell proliferation nor phosphorylation of ERK but demonstrated cell apoptosis by BDNF stimulation. BDNF increased cleaved-caspase3 and phosphorylation of JNK expression in OBA9 and these elevations were clearly diminished by JNK and p75 siRNA transfection.

CONCLUSION : These findings suggested that BDNF can activate p75-JNK-caspase3 signaling cascade in human gingival epithelial cells to induce cell apoptosis.

B-14 (23-8) Pharmacological Effects of General Anesthetics Altered by the Change of Subunit Composition of GABA$_A$ Receptors

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BACKGROUND : GABA$_A$ receptor is the main inhibitory receptor in the central nervous system and is molecular target of many general anesthetics. GABA$_A$ receptors comprise a heteropentameric protein complex assembled from 16 different subunits. The subunit constitution determines the pharmacological properties of the GABA$_A$ receptors. Therefore, genetically modified animals in a molecule related to the GABAergic neurotransmission exhibit different pharmacological responses to the anesthetic drugs. We have clarified that phospholipase C-related but catalytically inactive protein (PRIP) plays important roles in the intracellular transport of GABA$_A$ receptors.

OBJECTIVES : In this study, we investigated the pharmacological responses of anesthetic drugs in *Prip*-KO mice.

MATERIALS & METHODS : We homogenized the whole brain of *Prip*-KO and wild-type mice and fractionated into whole tissue fraction and plasma membrane fraction by centrifugation method. The expression of each subunit of GABA$_A$ and NMDA receptors in those fractions was analyzed by immunoblotting using each specific antibody. Propofol, etomidate, pentobarbital, and ketamine were intraperitoneally injected into *Prip*-KO and wild-type mice, and onset and duration time of loss-of-righting reflex were analyzed. Furthermore, mice were implanted with electroencephalogram and electromyogram electrodes for polysomnographic recordings. After recovery period, the mice were performed the polysomnography by administration of propofol and pentobarbital, and sleep-wake stages were analyzed.

RESULTS : Immunoblot analyses showed that the expression of β3 subunit of GABA$_A$ receptors was specifically decreased in the plasma membrane fractions of *Prip*-KO mice. Propofol- and etomidate-induced hypnosis were significantly decreased in *Prip*-KO mice, and sleep time measured by polysomnographic recordings was dramatically reduced in *Prip*-KO mice by administration of propofol.

CONCLUSION : Since the cell surface expression of β3 subunit of GABA$_A$ receptors was significantly reduced in *Prip*-KO mice compared with wild-type mice, the pharmacological effects of propofol and etomidate was significantly attenuated in *Prip*-KO mice. Therefore, PRIP may regulate the intracellular trafficking of GABA$_A$ receptor β subunit.

B-15 (23-9) Effect of Intermittent Parathyroid Hormone Administration on Osseointegration Aspects in Rabbit Osteoporosis Model

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BACK GROUND : Bone quality is an important predictive factor in the prognosis of dental implant. At low bone density sites such as found in osteoporosis patients, it is difficult to achieve favourable primary stability and osseointegration. Recently, intermittent parathyroid hormone administration is available to increase the bone density by enhancing the bone remodelling. Although parathyroid hormone administration could be possible treatment to improve osseontegration at dental implants, the details remain unknown.

OBJECTIVES : The aim of present study was to evaluate the effect of parathyroid hormone on the osseointegration in an osteoporosis model.

EXPERIMENTAL METHODS : Fifteen female New Zealand white rabbits (17weeks, 3.0-3.5 kg) underwent ovariectomy and administrated glucocorticoid to induce osteoporosis (OP). They were divided in three groups. The first group was injected parathyroid hormone intermittently for 4 weeks until implant placement (Gr. A) and the second group was injected parathyroid hormone intermittently for 8 weeks until end point (Gr. B). The remaining group was injected saline for 4 weeks until implant placement as control. After intermittent administration of parathyroid hormone or saline, titanium implants were inserted into the distal femur epiphyses of each animal. After 4 weeks of implant placement, implant stability quotient (ISQ) and removal torque (RT) were measured to evaluate the osseointegration.

RESULTS : ISQ values of Gr. B (80.9±1.9) were significantly higher than that of Gr. A (69.3±5.9) and control (66.6±5.3). RT values of Gr. B (77.0±29.2 Ncm) were significantly higher than that of Gr. A (36.0±8.4 Ncm) and control (31.6±15.2 Ncm).

CONCLUSION : In the limited results of this study, intermittent parathyroid hormone administration improves the placed implant stability in osteoporosis model. It is suggested that parathyroid hormone administration could be effective therapy to achieve the favourable osseointegration at low bone density sites.

B-16 (23-10) A Novel Isoform of FGFR2 Modulates FGF10-FGFR Signaling in Osteochondrogenesis

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The fibroblast growth factor (FGF) family consists of 22 members and plays a key role in many aspects of development including cell proliferation and differentiation through FGF receptor (FGFR) tyrosine kinases and their coupled intracellular signaling pathways. Gain-of-function mutations in the common extracellular domain of FGFR2 isoforms (type b and c) cause craniosynostosis and chondrodysplasia syndromes. FGF10, a major ligand for FGFR2b, is well known to regulate the proliferation of epithelial cells. Meanwhile, abrogation of FGF10 in an Apert syndrome mouse model rescues skeletal defects. Taken together with a possible role of FGF10 in chondrogenesis in vitro, these results lead us to hypothesize that FGF10-FGFR signaling is involved in bone and/or cartilage formation. To test this hypothesis, we generated transgenic mice overexpressing mouse FGF10 under the control of doxycycline. Transgenic (TG) pups overexpressing FGF10 from embryonic day 12.5 were smaller in body size and had shorter cranium in the rostro-caudal axis, compared with control littermates. Whole mount skeletal staining and micro-CT revealed skeletal dysplasia including dwarfed mandible and cleft palate in FGF10-TG fetuses. In analysis of a casual link between FGF10 and FGFR2, we found the additional alternative RNA splicing isoform of *Fgfr2b* expressed particularly in bone, cartilage, and mouse chondrogenic ATDC5 cells. The predicted translation product of this isoform appears to include the amino acid sequence encoded by exons 8 and 9 (type b and c, respectively) and lack membrane-spanning and tyrosine kinase domains. As expected, we confirmed that this isoform was translated as a soluble form of FGFR2b (sFGFR2b) in ATDC5 cells. Overexpressing sFGFR2b in ATDC5 cells increased cell proliferation, suggesting that sFGFR2b may act as a decoy receptor for FGFs. Thus, a large amount of FGF10 may trap sFGFR2b and allow for enhancing other FGF-FGFR signaling pathways, with resultant skeletal anomalies in FGF10-TG mice.

B-17 (23-11) TLR2 Plays a Key Role in *P. gingivalis*-induced NASH Progeression

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OBJECTIVES : Non-alcoholic steatohepatitis (NASH) develops from fatty liver. In addition, NASH has possibilities to develop into lethal diseases (cirrhosis and hepatic carcinoma). Therefore, prevention and intervention are needed. Recently, we reported that odonotogenic infection of *Porphyromonas gingivalis* (*P.g.*) exacerbated pathological progression of NASH. Moreover, we noticed that upregulation of TLR2 (*P.g.* -LPS receptor) in fatty livers strengthened sensitivity to *P.g.* -LPS. The aim of the study is to clarify the critical role of TLR2 signaling in pathological progression of NASH.

METHODS : ***In vitro* experiments:** human hepatocytes (HC-3716hTERT) induced fatty deposition by palmitate-treatment, were used as steatotic hepatocytes. We examined the effect of palmitate-induced steatosis on TLR2 expression. Moreover, the effect of TLR2 inhibitor on *P.g.* -LPS induced cytokines and inflammasome with or without palmitate-treatment. ***In vivo* experiments:** C57BL/6J (WT) mice and TLR2KO mice were used. They fed either chow-diet (CD) or high-fat diet (HFD) for 8 weeks and then half of the mice in each group were infected *P.g.* from pulp. HFD-*P.g.* (–), HFD-*P.g.* (+), CD-*P.g.* (–) and CD-*P.g.* (+) in each strain were prepared. Histological findings in liver were analyzed.

RESULTS : ***In vitro* experiments:** In steatotic hepatocytes, TLR2 mRNA and protein expression levels were upregulated. *P.g.* -LPS induced mRNA expressions of inflammasome (NLRP3 and caspase-1) and cytokines (IL-1β, IL-6, IL-8, MCP-1, TNF-α) were further upregulated in steatotic hepatocytes. Furthermore, TLR2 inhibitor intensively suppressed the expression of them. ***In vivo* experiments:** Histologically, in WT- HFD-*P.g.* (+) group, *P.g.* odonotogenic infection accelerated lipid deposition and macrophage infiltration compared with WT- HFD-*P.g.* (–) group. However, in TLR2KO HFD groups, the finding was not prominent.

CONCLUSIONS : Innate immune responses through TLR2 signaling play important roles in exacerbation of inflammation and lipid deposition by odonotogenic infection of *P.g.*. It is suggested that a possible novel therapy targeting TLR2 may be beneficial on NASH treatment.

B-18 (23-12) Oral Administration of Liposomal Bovine Lactoferrin Suppresses Progression of Rheumatoid Arthritis

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Bovine lactoferrin (bLF) is a multifunctional protein having modulatory effect of inflammation and immune response. Rheumatoid arthritis (RA) is autoimmune disease showing inflammatory bone destruction. It is well accepted that TNF-α is a key molecule for RA progression. Furthermore an imbalance of Th17 cells and regulatory T-cells is critical for RA progression. The purpose of this study is, therefore, to clarify the inhibitory effects of liposomal bLF (LbLF), which was designed to deliver bLF to small intestine, on RA progression.

In vivo EXPERIMENT : Mannan (RA inducer) was intraperitoneally injected to SKG mice (genetic RA model). Orally applied LbLF reduced joint swelling and inhibited bone destruction of joints. Histologically, pannus and osteoclastic bone destruction were suppressed in LbLF-applied animals. Moreover, flow cytometry analysis showed that orally applied LbLF improved the imbalance of Th17 cells and regulatory T-cells in splenocytes and lymphocytes. IL-17 production by Th17 cells of LbLF animals was significantly reduced.

In vitro EXPERIMENT : Anti-inflammatory effects of bLF were examined with human synovial fibroblasts from a RA patient (RASF) and macrophages (THP1), which are cytokine producing cells in pannus. Western blot and ELISA analyses showed that bLF pre-treatment suppressed TNF-α production from RASF and THP1 through inhibition of NF-κB and MAPK pathways. Moreover, immunoprecipitation analysis revealed that direct binding between bLF and TRAF2 (an adapter protein/ubiquitin ligase) was essential in bLF-induced TNF-α down-regulation.

CLINICAL TRIAL : 20 patients with RA were orally applied 270mg/day of LbLF for 3 months. Average of disease activity score 28 (DAS28) and CRP were gradually reduced and TNF-α and IL-17 levels were significantly downregulated by LbLF-supplementation.

We clarified that LbLF effectively prevented pathological progression of RA via suppressing TNF-α production and improving imbalance of Th17 cells and regulatory T-cells. Moreover LbLF-supplementation for RA patients showed improvement of RA. Therefore LbLF is a novel beneficial preventive/therapeutic reagent for RA.

B-19 (23-13) Development of Calcineurin Inhibitor Induced Gingival Hyperplasia Mice Model

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BACKGROUND : Drug-induced gingival hyperplasia (DiGH) is a side effect principally associated with three types of drugs: anticonvulsant (e.g. phenytoin), immunosuppressant (e.g. cyclosporine A) and various calcium channel blockers (e.g. nifedipine). Novel treatment method for DiGH is required because of recurrence after surgical treatment or difficulty of dose or drug changes. To develop novel treatment method, animal model with accurate and certainly occurred disease is necessary to elucidate the mechanism of DiGH. DiGH mice model is ideal for clarifying the mechanism because of the considerable background information, a wide range of genetically engineered strains and many high quality immunochemical and cellular reagents for experiments.

STUDY OBJECTIVE : The aim of this study is to develop the mice model and to elucidate of DiGH mechanisms.

EXPERIMENTAL METHODS : A sterile ligature (5-0 silk thread) was placed around the maxillary second molar of C57BL/6j mice (6- to 8-week-old). After a week, Cyclosporine A (CsA, 50 mg/kg/day), Tacrolims (FK506, 30 mg/kg/day) or Mizoribine (30 mg/kg/day) were injected to intraperitoneal for 4 weeks. The influence of bacterial infection on onset of DiGH was also examined by administration of antibiotics cocktail (ampicillin, metronidazole, vancomycin and neomycin sulfate). The degree of gingival hyperplasia was assessed by the percentage of gingival width of buccal side to width of second molar. The gingival tissue was histologically analyzed after HE staining.

ESSENTIAL RESULTS : Only the combination of ligature and calcineurin inhibitor (CsA or FK506) administration induced the gingival hyperplasia. Pretreatment with antibiotics suppressed the onset of gingival hyperplasia. In histological analysis, calcineurin inhibitor with ligature showed gingival connective tissue enlargement with increased collagen fibers.

CONCLUSIONS : The mice gingival hyperplasia model was successfully developed by the combination with ligature application (local bacterial infection) and calcineurin inhibitor administration. This model may lead to elucidate the mechanisms and be useful for development of novel treatment.

B-20 (23-14) Biological Effect of Carbon Ion and Gamma Ray Irradiation on Bone Metastasis of Breast Cancer Cells

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BACKGROUND AND RATIONALE : Periostin (POSTN) originally isolated as an osteoblast specific factor functions as a cell adhesion molecule. Overexpression of POSTN has been observed in the bone metastatic cancer. Many studies indicated that POSTN plays an important role in bone metastasis. Radiotherapy significantly improves local tumor control, but recent evidence suggests that radiation exposure to the tumor target can promote tumor invasion and metastasis. Irradiation to bone metastatic breast cancer cells induced expression of periostin increase in cancer cells. However, the role of POSTN in microenvironment of breast cancer cells invading area is still unclear.

STUDY OBJECTIVE : To examine the irradiation effects of carbon ion and gamma ray on POSTN expression in the breast cancer cells and bone invasion cytokine, such as Receptor activator of nuclear factor kappa-B ligand (RANKL) and Vascular endothelial growth factor (VEGF) in osteoblast cells.

MATERIAL AND METHOD : Breast cancer cells (FM3A/R) were exposed to carbon ion and gamma ray. Irradiated breast cancer cells were co-cultured with osteoblastic cells (MC3T3-E1). POSTN expression was examined in breast cancer cells, using the RT-PCR. Bone invasion cytokines, RANKL and VEGF were identified and isolated from osteoblastic cells, using the western blotting.

RESULTS : POSTN expression in breast cancer cells was increased within three days after irradiation of carbon ion and gamma ray. Carbon ion irradiated cells express POSTN less than gamma ray irradiated cells. The RANKL and VEGF of osteoblasts in co-culture assay were harmoniously enhanced with the expression of POSTN in breast cancer cells.

CONCLUSION : Radiotherapy often associated with risk of metastasis to bone that has not been overcome. Carbon ion irradiation could reduce the expression of POSTN in breast cancer cells compared to conventional gamma ray irradiation. These observations suggest that carbon ion irradiation may suppress the cancer invasion and metastasis to bone.

B-21 (23-15) The Differences of Electroencephalogram (EEG) Pattern during Sleep between Human Beings and Mice

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BACKGROUND : Loss of consciousness is one of the important end points in general anesthesia. Although EEG analysis is used as an index of loss of consciousness with either entropy® or BIS® monitoring during clinical general anesthesia, the differences of EEG pattern between human beings and animals have not been clear.

STUDY OBJECTIVE : We measured and analyzed EEG in mice, which are commonly used in animal experiments, and compared the analyzed EEG of mice with that of human beings to identify the validity of animal experiments for "drug discovery" of general anesthetics.

EXPERIMENTAL METHODS : We recorded both EEG and EMG in adult male ddY mice. Electrodes for EEG or for EMG were placed in anterior and posterior parts of skull or in muscle of neck under pentobarbital anesthesia, respectively. Recording time is 24 hours during both nighttime (20:00-08:00) and daytime (08:00-20:00).

Another experiment was performed to examine the effects of the intravenous anesthetic propofol on EEG and EMG. All data were analyzed using the software of Sleep Sign® (KISSEI COMTEC).

RESULTS : The summation of waking time in mice was significantly increased in the night time. There is no significant difference between the average of non-rapid eye movement (NREM) sleep and REM sleep time in the both day- and nighttime. After propofol injection, the increase in δ and θ wave bands and the reduction of EMG amplitude were observed.

DISCUSSION AND CONCLUSION : A significant increase in the summation of waking time during the night time suggested that mouse is nocturnal animal. The characteristics of sleep in daytime are similar to those in nighttime. This may be involved that mice follow polyphasic sleep patterns, while human beings follow monophasic sleep. These findings suggest that the characteristics of sleep in mice are different from those in human beings.

B-22 (23-16) A Rhizosphere Microbiota-derived Symbiotic Factor Prevents Intestinal Inflammatory Disease

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Our previous study showed one of the most frequently isolated species in plant rhizosphere microbiota, *Stenotrophomonas maltophilia*, can symbiotically inhabit cytosol compartment of murine bone marrow (BM) derived macrophages. The species produce a unique type II secretion system (T2SS) protein, smlt2713, which likely enhances anti-inflammatory IL-10 production by colonic macrophages under a MyD88-dependent manner. We also observed the *smlt2713* defective mutant is not able to inhabit cytosol compartment, instead makes aberrantly giant cohabitation niche in endosome/phagosome. Thus, we speculate that the smlt2713 protein should play a crucial role for the symbiotic cohabitation of the bacteria in intestinal macrophage and would contribute to create/maintain immunologically homeostatic environment in the intestinal mucosa. To explore *in vivo* effect of the *smlt2713* transduced macrophage on chronic inflammation, we devised the optimal expression system of the *smlt2713* gene in BM-macrophage, which preferentially produces IL-10 and exhibits other immunoregulatory phenotypes, and adapted the *smlt2713* transduced macrophage into murine $CD4^{+}CD45RB^{high}$ T-cell induced colitis model. Our immunopathological analysis revealed that intraperitoneal administration of the *smlt2713* transduced macrophage ameliorated inflammatory reactions in the colonic mucosa—infiltration of inflammatory cells and fibrosis of colonic mucosa as well as death caused by the chronic inflammation—developed by adoptive transfer of $CD45RB^{high}$ T cell population. Thus, the rhizosphere-derived symbiotic factor—smlt2713—might potentially provide a therapeutic for chronic inflammatory disease on the basis of an entirely unique mechanism.

B-23 (23-17) Bovine Lactoferrin Inhibits Oral Cancer Proliferation and Bone Invasion

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Lactoferrin (LF) has been reported to have potential effects on tumor growth. Some studies have shown the effects of LF on oral squamous cell carcinoma (OSCC), but its effects and mechanism remain to be clarified. We found new insights of effects of bLF and its subcellular mechanism, both *in vivo* and *in vitro* studies. *In Vivo*, 10^4 of SCCVII cells were transplanted to masseter region of C3H/HeN mice. The animals were orally administrated with liposomalized bLF (L-bLF) at 100mg/kgbw/day and 500mg/kgbw/day for 3 weeks. Micro-CT and histopathological observation showed that bLF significantly decreased tumor size and osteoclastic bone destruction of zygoma and mandible. *In Vitro*, bLF inhibited osteoclast differentiation in co-culture by using bone marrow cells. It was also demonstrated that bLF dowregulated expression of IL-1β and RANKL osteoclast inducers, and IL-1β-stimulated IL-1β and RANKL productions of OSCC by using RT-PCR and ELISA, through the inhibition of NF-kB and MAPK pathways. Furthermore, through LRP1, bLF significantly inhibited tissue plasminogen activator (tPA)-induced cells invasion by inactivation of ERK1/2 and some MMPs. Interestingly, bLF increased E-Cadherin expressions in both mRNA and protein levels, thereby inhibited EMT and induced its reverse program, mesenchymal-epithelial transition (MET), in the metastatic process, which then led an inhibition of cells migration and cells invasion. Our data suggested that bLF suppressed OSCC induced osteoclastogenesis, cells proliferation, migration, and invasion through LRP1. Thus bLF could be used as a preventive and supplemented agent for controlling OSCC.

B-24 (23-18) Odontogenic Infection of *Porphyromonas gingivalis* Exacerbates Pathological Progression of Non-alcoholic Steatohepatitis through Activation of Hepatic Stellate Cells by Macrophages

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Hepatic stellate cell activation by macrophages (MΦ) plays important roles in pathological progression of non-alcoholic steatohepatitis (NASH). Although we previously reported odontogenic infection of *Porphyromonas gingivalis* (*P.g.*) exacerbated inflammation and fibrosis in NASH, underlining mechanism is still unclear. In the present study, we investigated importance of MΦ-hepatic stellate cell interaction in *P.g.*-odontogenic infection induced pathological progression of NASH.

1) The cytokine expression in human MΦ (PMA-stimulated THP-1 cells) with *P.g.*-LPS stimulation was examined with/without palmitate-treatment. *P.g.*-LPS upregulated TNF-α and IL-1β expressions at mRNA and protein levels in both conditions. TLR2 (a *P.g.*-LPS receptor) inhibitor significantly inhibited *P.g.*-LPS induced cytokine production such as TNF-α and IL-1β. 2) Fibrosis-relating markers like α-smooth muscle actin were upregulated in hepatic stellate cells cultured with conditioned medium of *P.g.*-LPS stimulated MΦ, indicating that cytokines produced by *P.g.*-LPS stimulated MΦ contribute to hepatic stellate cell activation. 3) At 6-week after *P.g.*-infection from dental pulp of High Fat Diet (HFD)-induced fatty liver mouse model, effects of oral administration of antibiotic (azithromycin 100μg/body) with or without root canal treatment (azithromycin 50μg/ml) were examined to evaluate usefulness of therapeutic intervention. After 3 weeks, MΦ localization and fibrosis area were analyzed with immunohistochemical staining for Mac2 and Sirius red staining. The *P.g.*-odontogenic infection-increased MΦ foci (hepatic crown-like structures: hCLS), MΦ infiltrating area and fibrosis area were significantly reduced in treatment groups (hCLS; $P<0.01$, MΦ infiltrating area; $P<0.01$, fibrosis area; $P<0.05$).

Therefore, it is suggested that MΦ increased in the liver by *P.g.*-odontogenic infection exacerbates pathological progression of NASH through stimulating fibrogenic activity of hepatic stellate cell via excessive cytokine production. Moreover, elimination of *P.g.*-infection by dental treatment/antibiotic application may be a beneficial impact on NASH through reducing MΦ in the liver.

Poster Presentation

B-25 (23-19) MEPE-ASARM, a Substrate of PHEX, Decreases Bone Volume Independently of Serum Phosphate Levels

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BACK GROUND : Bone matrix protein MEPE (matrix extracellular phosphoglycoprotein) is involved in phosphate metabolism and contains the acidic serine- and aspartate-rich motif (ASARM) that is produced by proteolytic processing and released into the extracellular compartment. Phosphorylated ASARM (pASARM) is degraded by phosphate regulating endopeptidase, X-linked (PHEX), which is expressed in osteoblasts and odontoblasts. Loss-of-function mutation in PHEX causes bone and tooth mineralization defects. Therefore, the accumulation of pASARM in bone with loss-of-function mutations in PHEX may cause mineralization defects. Recently, we showed that pASARM inhibited matrix mineralization in osteoblast cultures, and PHEX partially rescued the effect.

OBJECTIVES : In this study, we examined the effect of exogenously added pASARM on bone metabolism in vivo.

EXPERIMENTAL METHODS : We administrated ASARM, pASARM or vehicle alone to C57BL/6 male mice (6-week-old) for two weeks via micro osmotic pumps. After administration, several bone parameters were measured by μCT. Serum and urine biochemical analysis were performed. mRNA levels related in bone and phosphate metabolism were determined by real time PCR. Bone histomorphometry were performed by calcein double labeling and villanueva staining.

RESULTS : pASARM did not impinge on body weight, serum calcium and phosphate levels, and renal *Slc34a3* and *Cyp27b1* mRNA levels. In parallel with these, pASARM did not change urine calcium and phosphate levels. Neither gene expression profiling in bone nor serum markers showed significant effects of pASARM on bone formation and resorption. Interestingly, however, μCT analysis indicated that pASARM decreased tissue volume, bone volume, tissue surfaces, bone surfaces and trabecular thickness and increased trabecular bone pattern factor, with a concomitant decrease in calcein labeling of bone surfaces. Because non-phosphorylated ASARM has little effect on bone, the phosphorylation is necessary for ASARM to exert its actions in bone.

CONCLUSION : These results suggest that pASARM may inhibit bone mineralization without changes in serum phosphate and calcium levels.

B-26 (23-20) *Porphyromonas gingivalis* Infection Exacerbates Rheumatoid Arthritis in Experimental Mice Model

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BACKGROUND : The epidemiological studies have revealed the relationship between periodontitis and rheumatoid arthritis (RA). However, the detail of the mechanism is still unclear. Recently, *Porphyromonas gingivalis* (*Pg*) was reported to produce citrullinated protein (CP), a causative factor of RA.

STUDY OBJECTIVE : To clarify the role of *Pg* infection on the enhancement of RA in mice model.

MATERIALS AND METHODS : RA model mice (SKG mice, CLEA Japan) were established by intraperitoneal injection of laminarin (LA). Mice were divided into 4 groups [(A) PBS injection, (B) LA injection, (C) *Pg*+LA injection, (D) *Pg* injection]. The joint swelling was evaluated by arthritis score. The bone morphology was observed by micro CT. The ankle joint tissue was stained with hematoxylin and eosin. Anti-cyclic-citrullinated protein (CCP) antibody and matrix metalloproteinase-3 (MMP-3) in serum were measured by ELISA. The cytokine profile in serum was analyzed by Bio-Plex system (Bio-Rad). Osteoclast differentiation of bone marrow cells (BMCs) from leg bone in each group of mice was examined. We also examined the localization of *Pg* and the presence of CP in joint tissue by Western blotting.

ESSENTIAL RESULTS : In group C, arthritis score was 3-fold high compared to group B. The micro CT image in group C clearly showed the decrease of bone density at joint of ankle. The infiltration of inflammatory cells and the bone destruction were also observed in ankle tissue. Anti-CCP antibody and MMP-3 production in serum from group C were highest among the groups. The levels of IL-2, IL-6, CXCL1 and MIP-1α in serum from group C mice was significantly high compared with the other groups. The osteoclastogenesis of BMCs was increased in group C mice. Furthermore, in group C, *Pg* components and higher protein level of CP were detected in joint tissue.

CONCLUSION : *Pg* infection has a potential for RA exacerbation.

B-27 (23-3) Irradiation Effects of Newly Developed Low Temperature Multi Gas Plasma Jet on Oral Bacteria

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BACKGROUND AND RATIONALE : It is important to consider oral hygiene during orthodontic treatment as oral tissue and teeth are subjected to long term compromised oral environment due to use of various types of orthodontic appliances and become susceptible to plaque, caries, gingivitis. Plasma sterilization has attracted attention in dental community for low frequency pressure plasma jets in order to sterilize the infected dental tissue.

STUDY OBJECTIVE : We carried out experiments to evaluate the sterilization effects of this technology on oral pathogenic microorganisms (*S. mutans, Lactobacillus*, *A. Actinomycetemcomitans*) to determine its potential for clinical application.

DESIGN AND EXPERIMENTAL METHODS USED : Experiments were conducted using a newly developed low temperature multigas plasma jet. We used O^2, N^2, Ar and (O^2, 50%+N^2, 50%). The microbes were cultured in BHI broth. Plasma irradiation time were 0 (control), 10 and 60s from 2 and 20mm on the agar plates. CFU was counted after 24 hours. Next, 24 well plates were filled with 50µl of microbial suspension with 450µl of citrate buffer (pH 3.5, 5.0, 7.5). Then, plasma was applied for 0 (control), 1and 5 min. After irradiation, CFU was counted after 2 days. The 3rd experiment involved direct application of plasma on human extracted tooth. Plaque was initiated on the enamel surface of tooth before applying plasma. Plasma irradiation time was 60s and continued for 21 days. The depth of enamel resorption area was noted by a nanoscale hybrid microscope.

ESSENTIAL RESULTS : O^2 had the best sterilizing effect and 60sec irradiation time from 20mm was significant. In the liquid phase sterilizing effect became prominent at pH 3.5. Decalcification of enamel was noted significantly lower in plasma irradiated tooth surface compared to control.

CONCLUSION : The experiments revealed that multi gas plasma jet had best sterilizing effect with O^2 plasma on microorganisms and it has great potential to be used for dental treatment.

B-28 (24-1) A Novel Intragenic Microdeletion in *RUNX2* in a Chinese Family with Cleidocranial Dysplasia

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BACKGROUND : Cleidocranial dysplasia (CCD) is a rare human autosomal dominant skeletal dysplasia characterized by cranial, clavicular, and dental anomalies. CCD is usually caused by mutations in the runt-related transcription factor 2 gene (*RUNX2*), which was mapped to chromosome 6p21. However, the pathogenesis of CCD is always different in different races. Most of the data on *RUNX2* mutations in CCD patients have been generated from Caucasian populations, and few *RUNX2* mutations have been reported in Chinese CCD patients. And the stringent genotype-phenotype correlations also have not been found to date.

OBJECTIVES : Present study was designed to investigate the aberration in the *RUNX2* gene in a Chinese family with CCD.

EXPERIMENTAL METHODS : Clinical evaluations were performed on all participants in the CCD family by radiographic techniques. Genomic DNA was isolated from the blood samples of all 11 participants in this family, including 3 patients. Mutation analysis of *RUNX2* was performed using amplified polymerase chain reaction and direct sequencing. To determine the copy number of the exons of *RUNX2*, real-time quantitative PCR was performed for all 3 patients and the 8 unaffected individuals.

RESULTS : A previously reported SNP (rs6921145) was found, but no causative mutation was detected in the coding regions of *RUNX2* by direct sequencing analysis. Real-time quantitative PCR revealed a novel exon 4 to exon 7 intragenic deletion in *RUNX2* in all 3 affected family members.

CONCLUSION : Our findings suggest that the intragenic deletion from exon 4 to exon 7 in the *RUNX2* gene is the cause of CCD in this family and most likely causes CCD by altering the protein structure of RUNX2, which then fails to regulate the transcription of *RUNX2*-regulated genes. We therefore highlight the importance of considering deletions and duplications in patients without a causative mutation identified by DNA sequencing.

C-1 (03-1) Effect of Aging Treatment on the Flexural Strength of Implant Provisional Restoration Materials

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OBJECTIVES : The aim of this study was to investigate the mechanical properties of resins for implant provisional restoration. After aging treatment on some kind of resins and CAD/CAM PMMA block for Implant provisional restorations flexural strength, flexural modulus was measured and the fracture surface of specimens was observed.

METHODS : Flexural strength and flexural modulus of Vertex self curing resin, Luxatemp automix solar and Dmax PMMA block was measured by UTM. The fracture surface of specimens was observed by SEM.

RESULTS : 1. Flexural strength and flexural modulus values decreased in the order of Dmax PMMA block, Luxatemp automix solar, Vertex self curing resin and significant differences were found ($p<0.05$).

2. Flexural strength values of the experimental group to dried for 24 hours at 37°C and the experimental group to immersed in 37°C distilled water for 14 days, Vertex self curing resin and Luxatemp automix solar showed decreases of the flexural strength values. Dmax PMMA block showed increases in the flexural strength valuse. Vertex self curing resin and Dmax PMMA block showed significant differences ($p<0.05$).

3. Flexural modulus values of the experimental group to dried for 24 hours at 37°C and the experimental group to immersed in 37°C distilled water for 14 days, flexural modulus values decreased in all experimental groups. Vertex self curing resin and Dmax PMMA block showed significant differences ($p<0.05$).

4. In the observation of fracture surface of specimens, the difference between the experimental group to dried for 24 hours at 37°C and the experimental group to immersed in 37°C distilled water for 14 days did not appeared.

CONCLUSIONS : Among experimental groups, CAD/CAM PMMA block was significantly highest flexural strength and flexural modulus values followed by dual curing resin, and self curing resin ($p<0.05$). After aging treatment, self curing resin and dual curing resin showed decreases in the flexural strength, but CAD/CAM PMMA block showed increases in the flexural strength. All experimental group (self curing resin and CAD/CAM PMMA block) except for dual curing resin showed significant differences by aging treatment ($p<0.05$). In the observation of fracture surface of specimens, there were no apparent differences by aging treatment.

C-2 (07-6) Effects of Different Beverages on Color Stability of Heat-cured Acrylic Resin

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Discoloration of dental materials, especially for denture base, is one of the causes of patient dissatisfaction and time expense for replacement the new one. Therefore, the study to determine the color stability of heat-cured denture base acrylic resin (Meliodent®) after immersed in different kind of beverages for a period of time were observed. Fifty heat-cured denture base acrylic resin disc specimens (50.0 mm diameter, 0.5 mm thick) were prepared, then stored in distilled water at 37°C for 24 hours. Color of all specimens was measured with a spectrophotometer using CIE L*a*b* relative. The specimens were divided into 5 groups (n=10) and then stored in 5 different kind of beverages: vegetable and fruit juice, coffee, green tea, carbonated drink and distilled water as a control for 30 and 45 days. After storage, the color of the specimens was measured again and color changes (ΔE) were calculated. Mean data of all groups were compared with One-Way ANOVA and multiple comparisons by Bonferroni test. The result of 30 days immersion showed no significant different in mean color changed ($p>0.05$) with vegetable and fruit juice group had the most color changed. By the way, the result of 45 days immersion the most color changes was the vegetable and fruit juice group, followed by coffee, carbonated drink, green tea and then distilled water group (10.77, 10.01, 9.30, 8.90, 5.71) with statistically significant different ($p=0.00$). When compared to clinical acceptable value of National Bureau of Standard units ($\Delta E>3.3$), all specimens both 30 days and 45 days immersion color changed were exceeded. Avoidance or minimize of drinking colored beverages such as green tea, carbonated drink, coffee and vegetable and fruit juice may be suggested for denture wearing patients to keep satisfaction color stability of denture base.

C-3 (07-7) Anti-adhesion Efficiency of *Candida albicans* on Curcumin Coated Heat-cured Acrylic Resin by Polyelectrolyte Multilayer Technique

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BACKGROUND : Post-surgical maxillary defects can be immediately reconstructed using an acrylic resin obturator to restore function and esthetics in maxillofacial defects. Long-term use of acrylic resin obturator can accumulate microorganisms resulting in biofilm formation such as *Candida* biofilm, which is initially a pathogenesis of infections in the oral cavity of immunocompromised or poor oral hygiene patients. Currently, there have been many studies to ameliorate anti-microbial properties of acrylic resin. The fabrication of polyelectrolyte multilayer (PEM) films is one of surface modification techniques using Layer-by-Layer (LbL) deposition of oppositely charged polyelectrolytes. Several reports showed good properties of curcumin for examples, anti-microbial, anti-inflammation and healing effects. However, there is no recent study of curcumin as surface modifying agent on acrylic resin coated via PEM technique.

OBJECTIVE : This study evaluated the inhibitory effect of curcumin PEM films coated heat-cured acrylic resin on the adhesion of *Candida albicans*.

EXPERIMENTAL METHODS : The heat-cured acrylic resin samples (3x6x1mm) were prepared and coated with curcumin at concentrations of 0.01, 0.1, 1 and 10 mM by PEM technique. The adhesion of *Candida albicans* was evaluated by adhesion assay and cell adhesion morphology was examined by scanning electron microscopy (SEM) analysis.

RESULTS : The uncoated acrylic resin discs had significantly more microorganism adhesion (100±0%) than those coated with curcumin at concentration of 0.01 (56.58+5.91%), 0.1 (39.44±0.68%), 1 (38.50±1.50%) and 10 mM (29.24±2.52%), respectively ($p<0.05$). In addition, increasing concentrations of curcumin showed a significant reduction in the percentage of microorganism adhesion. Moreover, the result form SEM analysis was corresponded to the adhesion assay.

CONCLUSION : The curcumin coated acrylic resin by PEM technique reduces the adhesion of *Candica albicans* and can be further used to reduce infectious complication in patients with maxillary defects.

C-4 (09-3) Antifungal Activity of Type III Dental Gypsum Incorporated with 3-iodo-2-propynyl-butylcarbamate

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BACKGROUND : The growth of fungi on dental model can damage the gypsum and affect the physical appearance of the dental model. Moreover, it can be transferred among patients and dental personnel. Fungi also relate to numerous acute and chronic illnesses. Thus, the development of antifungal dental gypsum is required to avoid the fungal growth on the dental model.

OBJECTIVE : This study aims to evaluate the antifungal activity of type III dental gypsum incorporated with 3-iodo-2-propynyl-butylcarbamate (IPBC).

EXPERIMENTAL METHODS : The antifungal activities of type III dental gypsum (The Siam Moulding Plaster Co., Ltd, Thailand) incorporated with 0.005% w/w IPBC were tested against *Penicillium notatum* MI-311 and *Aspergillus flavus* MI-321 obtained from culture collection of Oral Microbiology Department, Faculty of Dentistry, Mahidol University, Thailand, and *Aspergillus spp*. isolated from dental model of orthodontic patient using technique modified from ASTM G21. Type III dental gypsum was prepared and poured into the perti dishes and allowed to set for 30 min. Fifty µL of spore suspension of each fungus (10^4 CFU/mL) was dropped on the surface of gypsum and incubated at room temperature, ≥85% relative humidity for 28 days. Fungal growth was visually scored according to ASTM G21 compared with the control group (gypsum without IPBC).

RESULTS : Type III dental gypsum incorporated with IPBC showed a significant antifungal activity against all tested fungi. No fungal growth was found on the tested gypsum, as opposed to the control group which showed score 3 and 4 of fungal growth.

CONCLUSION : Type III dental gypsum incorporated with 3-iodo-2-propynyl-butylcarbamate (IPBC) had antifungal effect on all tested fungi under the environment of the experiment. This developed antifungal dental gypsum could be used to fabricate dental models, which can be preserved overtime for the purpose of patients' treatment planning and evaluation.

C-5 (14-1) Gene Transfection with the Nanohydroxyapatite-collagen Scaffold, Containing DNA-functionalized Calcium Phosphate Nanoparticles

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BACKGROUND : Numerous scaffolds have been developed and reported successful tissue regeneration in combination with growth factors. However the activity and the concentration of growth factors locally is not enough in the later stages of tissue repair process, since the duration and volume of release of growth factor mainly depends on the based scaffold graduation. The scaffold including gene transfection agents loaded scaffold might make the cells invade the scaffold and take up the agents into the cytoplasm; this will induce the cell to release the objective growth factor with activity.

STUDY OBJECTIVE : This study aimed to fabricate the gene releasing biodegradable scaffold for bone regeneration using gene transfection vector and investigate the transfection efficiency.

METHODS : For the preparation of calcium phosphate vector (hereafter called as CaP), a dispersion of $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ were mixed with DNA plasmid encoded human BMP-2 and protamine complex and finally, accomplished by the addition of either an aqueous solution of protamine, polyethyleneimine and Octa-arginine. For the preparation of nanohydroxyapatite-collagen (hereafter called as nHAC), type I atelocollagen gel was dissolved in 0.01 M HCl and added KH_2PO_4/K_2HPO_4 and Tris-buffer with $CaCl_2$ solution at 4˚C. Finally, CaP add into nHAC and accomplished by freeze-drying process for 12 hours (0.08mbar, -80˚C). The transfection efficiency and cell viability of nCaP/nHAC was investigated on human MSCs by ELISA test.

RESULTS : The scaffolds had high biocompatibility and succeeded to transport DNA into nucleus of attached cells. Especially, the scaffold with the CaP vector containing protamine showed the highest biocompatibility and transfection efficiency.

CONCLUSION : We demonstrated the successful gene transfection of DNA-functionalized calcium phosphate nanoparticles loaded on nanohydroxyapatite-collagen scaffolds. This modified nHAC scaffold can serve as an efficient gene releasing tool in tissue engineering for treating various bone defects.

C-6 (16-1) Investigation on Orthodontic Brackets Strength Differences Based on The Design and Materials Simulation

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INTRODUCTION : In Indonesia today ortodontic brackets are still imported from other countries, they are not well suited with the Indonesian anatomical tooth. This condition was viewed from the contour of the labial teeth, tooth size and the angle of inclination teeth. The proper brackets design would deliver force from arch wire to the teeth, then moved to the desired position. In order to produce an orthodontic brackets that suited with the Indonesian people, therefore it was necessary to investigate the imported brackets in order to acknowledge the advantage and disadvantages of the design and materials.

OBJECTIVES : The purpose of this study is to investigate the strenght of two different bracket designs in receiving force from the archwire through computer simulation.

MATERIAL DAN METHODS : Two types of imported stainless steel orthodontic brackets with different designs (Mini Sprint Forestadent (Roth) (A) and Geminy 3M (B) with slot 0.022 x 0.028 inch (Roth) were used in this study, the brackets were redrawn using Autocad Inventor software 2013®., then simulated by gived a pressure of 80 g of archwire (0.019 x 0.025 inch and a length of 9 mm) to the two sides of the wing brackets in the opposite direction. this situation was analogous as the archwire deflection when expecting to move teeth tipping in the oral environment.

RESULT : When the wing parts of orthodontics bracket received a force in the opposite direction, Bracket A receives pressure force that was 42.75 MPa larger than the Bracket B was 21.47 MPa. This differences evident were described in the simulation, which shows the color indicators on the brackets.

CONCLUSION : The orthodontic brackets strength is shown in the concept of design and the selected material. But this software has limitations in selecting materials which well suited with the original product, therefore the simulation can only viewed from the design of the brackets. The main differences of the two brackets design are shown on wing and the size of brackets. The more larger the brackets size then the force received can be divided into a large area and would minimized the stress. The results of this investigation also showed that the failure of the production process also shows influence to the designs precision and orthodontic brackets strength.

D-1 (01-4) Health Education Strategies to Improve Preschooler's Parents Behaviour in Oral Health

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BACKGROUND : The prevalence of dental caries among preschooler is still high in Indonesia. Lack of parents' behaviour has contributed to the rise of dental caries prevalence because preschooler still needs help from their parents in oral health care. During this time, oral health education is carried out only for children. The new oral health education strategy to improve preschooler's parents' behaviour in oral health need to be developed.

OBJECTIVES : To explore health education strategy in improving preschooler's parents' behaviour in oral health.

DESIGN : This was a literature review. Articles published from 2005 to 2015 in English and indexed in SAGE, EBSCO host, Science Direct, Online Wiley Library, and ProQuest databases were searched by using the key term oral health, oral health education, oral health behaviour, and parents of preschool children. Finally, the Google Scholar search engine was used to do a comprehensive search to ensure completeness of the search. Only studies that indicate a significant change after oral health education on parents behaviour in oral health were reviewed.

RESULTS : The search yielded 7 articles: randomised controlled trial (3); quasy experiment (2); pre experiment (1); and comparative (1). The longest intervention was conducted five years (since birth until 5 years old). The provider of health education were dentist, dental hygienist, health volunteers, and interprofessionals (includes nurses). The type of health education strategy were home visit and consultation, lecture, and hands on training on brushing child's teeth. The type of media used were printed materials (booklet, brochure, pamphlet), video, and mobile phone text messaging. Material on oral health education includes oral hygiene habit, dietary habit, and dental attendance pattern.

CONCLUSION : Rigorous study about health education strategy in improving preschooler's parents' behaviour in oral health is scarce. Future study can use these findings to develop effective health education strategy to improve preschooler's parents' behaviour in oral health.

Key words : health education, oral health behaviour, preschooler's parents

D-2 (01-5) Literature Review: Oral Health in Pregnant Women

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BACKGROUND : Oral health problem usually happened during pregnancy. It is important to improve oral health in women with pregnancy to prevent serious dental disease during pregnancy (Bansal and Gupta, 2013). However maintaining oral health in pregnancy hadn't be the main concern of women and family. Health care professional including nurses and midwives should build partnership with dentist to improve oral health in pregnant mother.

OBJECTIVES : The purpose of this literature review was to identify women behavior in maintaining oral health during pregnancy and women oral health status.

METHODS : A literature research was conducted through Portal Garuda and Google Scholar database using the key words pregnancy, pregnant women, and oral health. The literature research use articles between 2010 to 2015. There were 10 articles and four was reviewed.

RESULTS : A study at medan suggested that pregnant women have lack of knowledge about maintaining oral health in pregnancy and only 8% had visited dentist (Diana and Hasibuan, 2010). An observational research using dental examination showed that caries prevalence on pregnant women was 41.9% with the average of decayed, missing, and filled teeth (DMFT) index was 6.44 (Lestari, 2012). Other study conduct on 30 pregnant women in Puskesmas jember found that less than 50% of women answer correctly about periodontal disease but found that 92.2% answer correctly on taking care of mouth and dental health; and found 56.7% had periodontal disease (Saadiyah, 2014). On contrary a study at Puskesmas Bahu Menado on 50 pregnant women found that pregnant women had good knowledge, attitude, and action on oral health care (Arisanty, 2013).

CONCLUSION : Periodontal problem during pregnancy still high and pregnant women awareness about periodontal disease and treatment still low. Health care professional involvement in improving pregnant women awareness of their oral health is inevitable.

D-3 (02-1) An Experience of Odontogenic Cysts and Tumors of the Jaw in Eastern Nepal

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BACKGROUND AND RATIONALE : Odontogenic cysts and tumors are those arising from the tooth forming elements of the jaw. The prevalence, nature and site of predilection of these lesions are variable. The basic information of these lesions are very important to know as it best describes the nature and trend of these diseases and its impact on human health.

OBJECTIVE : To survey the characteristics of odontogenic cysts and tumors of the jaw.

DESIGN AND EXPERIMENTAL METHODS USED : This was a retrospective study and involved collection of data from 1998-2012; pertaining to odontogenic cysts and tumors of jaw from the archives of department of pathology. The demographic and clinico-pathological data were collected and entered in MS Excel sheet 2000 and subjected to descriptive statistical analysis.

RESULTS : A total of 2767 cases were encountered of the oral and maxillofacial region of which 6.14% (170 cases) were of odontogenic origin. 61.7% and 38.3% were odontogenic cysts and tumors respectively. More females showed predilection for odontogenic cysts (52.38%) and tumors (69.23%) as compared to males (47.61 % & 30.76% respectively). Among the odontogenic cysts, radicular was the most frequent (52.38%) followed by dentigerous cysts (29.52%). The most frequent odontogenic tumors were of ameloblastoma (35.38%) followed by keratocystic odontogenic tumor (18.46%). Follicular followed by plexiform was the most frequent histopathological variant of ameloblastoma. Both odontogenic cysts and tumors occurred during the second to fourth decade of life with posterior mandible being the most common site.

CONCLUSION : This study suggests radicular cyst and ameloblastoma to be the most frequent odontogenic cyst and tumor. All the lesions diagnosed are benign lesions. Both odontogenic cysts and tumors were found to be more prevalent in females compared to males. With the lack of information regarding the trend of cysts and tumors of odontogenic origin of the jaw in Nepal, this study attempts to highlight the nature and behavior of the pathology.

SESSION SUB-THEME : Oral Health and Clinical Treatments

D-4 (04-3) Antifungal Effect of Chitosans on Clinical *Candida* Isolates

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BACKGROUND : Oral candidiasis is a common opportunistic infection. Since the emergence of antifungal drug resistant strains, a new antifungal agent is required. Chitosans are deacetylated derivatives of chitin which is the exoskeletons of Crustaceans. They have antimicrobial effects, biodegradable, biocompatible, and low toxic.

OBJECTIVE : To examine an antifungal activity of high molecular weight (HMWC), N-succinyl (NSC), oligomer and polymer chitosans against clinical *Candida* isolates.

METHODS : Minimal fungicidal concentrations (MFC) of four chitosan derivatives; HMWC (150-200 kDa), NSC, oligomer (7-9 kDa; 80 mesh) and polymer (900-1,000 kDa; 40mesh) were investigated by using agar dilution method. In the same time, minimum inhibitory concentration (MIC) of two antifungal drugs; amphotericin B (AP) and fluconazole (FL), against clinical *Candida* isolates were determined by Epsilometer test (E-test).

RESULTS : The MFC of HMWC was different among species, even among strains within range 2.5-5 mg/ml. The highest concentration of NSC (4 mg/ml) was unable to kill any clinical isolated strains. Oligomer and polymer chitosans had an antifungal activity towards majority of oral *Candida* species with variation. The MIC of AP was range from 0.032 to 6 mg/ml. There were 4 strains of *C. parapsilosis* and 1 strain of *C. glabrata* showed resistant to AP (MIC ≥ 2 mg/ml). Fluconazole had the MIC range between 0.032-24 mg/ml against all tested isolated strains except *C. krusei* that has natural resistance to the drug (MIC ≥ 64 mg/ml). Interestingly, HMWC with the concentration between 2.5-5 mg/ml can kill most antifungal drug-resistant strains of *C. parapsilosis*, *C. glabrata* and *C. krusei*.

CONCLUSION : HMWC has an antifungal activity against clinical *Candida* isolates. Moreover, it can kill AP- or FL-resistant strains that found in the study. This finding indicates a possibility to develop HMWC as a synergistic antifungal agent against antifungal drug-resistant strains. However, further studies on synergistic effect should be continued.

D-5 (06-1) Correlation between the Pharyngeal Airway Space and Head Posture After Surgery for Mandibular Prognathism

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PURPOSE : The aim of this study was to determine the correlation between the pharyngeal airway space and head posture after mandibular setback surgery for mandibular prognathism.

MATERIALS AND METHODS : Serial lateral cephalograms of 37 patients with mandibular prognathism who underwent intraoral vertical ramus osteotomy (IVRO) were evaluated before (T1) and immediately (T2), between 6 weeks and 3 months (T3), and more than 1 year (T4) after surgery. Paired t-tests and Pearson's correlation analysis were used to evaluate the postoperative changes in all cephalometric parameters, including the mandible, hyoid, head posture (craniocervical angle), and pharyngeal airway space.

RESULTS : The mandible and hyoid were setback by 12.8 mm and 4.9 mm, respectively, at T2. Furthermore, the hyoid showed significant inferior movement of 10.7 mm, with an 8-mm increase in the tongue depth. The upper oropharyngeal airway (UOP) shortened by 4.1mm, the lower oropharyngeal airway (LOP) by 1.7 mm, and the laryngopharyngeal airway by 2 mm. The craniocervical angle showed a significant increase of 2.8°. UOP and LOP showed a significant correlation with the craniocervical angle at T2 and T4.

CONCLUSIONS : Our findings conclude that the oropharyngeal airway space is significantly decreased and correlated with a change in the head posture after mandibular setback surgery.

D-6 (07-8) Treatment of Squamous Papilloma with Double Pedicle Flap and Subepithelial Connective Tissue Graft: A Case Report

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BACKGROUND : Oral squamous papilloma (OSP) is a benign lesion that shows the proliferation of stratified squamous epithelium related to human papilloma virus (HPV) subtypes 6 and 11. Surgical removal is the treatment of choice. Complete removal of OSP may result in mucogingival dehiscence defect which can cause esthetic problems, hypersensitivity and ineffective oral hygiene care.

OBJECTIVE : This report describes a combination of two surgical procedures: (1) excisional biopsy of OSP in the maxillary anterior teeth and (2) double pedicle flap with subepithelial connective tissue graft to restore the defect after complete removal of OSP.

METHODS : A 61 year old Thai female patient presented with a rubbery, pink-to-white, cauliflower-like, asymptomatic, 3 x 3.5 mm lesion that extends from the gingival margin of the maxillary left central incisor to mucogingival junction. The lesion was completely excised resulting in a mucogingival dehiscence defect. A double pedicle flap with subepithelial connective tissue graft was performed to treat the defect. The excised tissue was sent to Department of Pathology. The patient was seen 10 days post-op for suture removal and oral hygiene instructions. Follow-up assessments and professional dental cleaning were conducted every month for up to 6 months.

RESULTS : At 6 months, clinical follow-up showed complete coverage of the surgical site. No recurrence of the lesion was observed. The biopsy result revealed squamous papilloma.

CONCLUSION : Treatment of OSP with surgical removal may result in a defect in the gingiva. Double pedicle flap with subepithelial connective tissue graft should be considered as an alternative treatment to manage the defect especially in the esthetic zone.

D-7 (07-9) Clinical Management of Pyostomatitis vegetans: A Case Report

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Pyostomatitis vegetans (PV) is a rare oral disorder first introduced by McCarthy, who believed the lesion to be the oral counterpart of pyoderma vegetans, a disease characterized by pustules, erythema and brown annular vegetating plaques affecting mainly the intertriginous areas resolving with post-inflammatory hyperpigmentation. PV is considered a highly specific marker for inflammatory bowel disease (IBD) particularly ulcerative colitis. The disease is most commonly found in young and middle-aged adults with M:F ratio = 3:1. In most cases, bowel disease precedes the onset of oral lesions by months or years but the symptoms may be minimal and can go undetected. The pathogenesis is as yet unknown although immunological disorders, microbial factors and nutritional deficiency have been suggested as possible etiologic factors.

This case of a 54-year-old woman presented with multiple friable pustules on both maxillary and mandibular attached gingiva was reported. These pustules were painless, but when they ruptured, areas of ulceration causing some discomforts were observed. This patient also reported some mild symptoms of bowel disease. A biopsy from the maxillary right palatal gingiva was performed. Histopathological findings consist of numerous intraepithelial and subepithelial microabscesses containing a large number of neutrophils and eosinophils within the stratum spinosum and in the connective tissue papilla areas. Finally, the definitive diagnosis of Pyostomatitis vegetans was made.

Management of PV, in the absence of IBD, therapy with topical corticosteroids can be successful but systemic corticosteroids, dapsone and sulphasalazine may also be useful. Treatment of any associated bowel disease may be effective in controlling oral and skin lesions but lesions may recur after treatment is reduced or stopped. In this case, our treatment includes a combination of systemic corticosteroid (Prednisolone), topical corticosteroid (Fluocinolone acetonide using customized trays) and Metronidazole. Spontaneous remission occurred after 6 months of treatment when the symptoms of IBD were resolved.

D-8 (07-10) Treatment of Gingival Recession with Subepithelial Connective Tissue Graft Combined with Double Papillae and Lateral Sliding Flap: A Case Report

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BACKGROUND : Gingival recession is a multifactorial periodontal problem that results in aesthetic deficiency and hypersensitivity. According to many systematic reviews, subepithelial connective tissue graft is a treatment that has the highest success rate. Thin keratinized tissue is more prone to inflammation. There are various techniques to gain the keratinized tissue.

OBJECTIVE : The purpose of this case report is to present the treatment of gingival recession with inadequate keratinized tissue at the same time by using subepithelial connective tissue graft combined with double papillae and lateral sliding flap.

METHOD : This case report is on a 22-year-old Thai male patient with a chief complaint of aesthetic deficiency on tooth 23 and 24 after he had orthodontic treatment about 8 years ago. Clinically, 3-4 millimeters of gingival recession was present on the buccal aspect of tooth 23 and tooth 24. No loss of proximal support on both teeth. Keratinized tissue on tooth 24 was 1 millimeter. No hypersensitivity was detected. Class I Miller's classification of gingival recession on tooth 23 and 24 were observed. The patient was treated with subepithelial connective tissue graft harvested from the palate combined with partial thickness double papillae to cover recession of tooth 23 and lateral sliding flap to cover recession of tooth 24.

RESULT : 10 weeks post-op, result showed complete root coverage on both teeth with similar colour to adjacent area. The zone of keratinized tissue on tooth 24 increased by 4 millimeters. The gingival thickness was also increased clinically comparing to non-grafted area.

CONCLUSION : Miller class I gingival recession has a predictable outcome for complete root coverage when treated with connective tissue graft. To gain keratinized tissue simultaneously with root coverage, double papillae and lateral sliding flap should be taken into consideration when the interdental papillae and keratinized tissue of the adjacent tooth are available.

D-9 (07-11) Effect of Silver Diamine Fluoride, CPP-ACP, Fluoride Varnish on Surface Microhardness of Human Dental Enamel

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BACKGROUND : Calcium fluoride is a major reaction product formed during topical treatment of dental hard tissue by various fluoride containing topical materials, and acts as a reservoir of fluoride. To date, there is no available data on silver diamine fluoride as compared to the other materials, regarding its effect on surface microhardness.

OBJECTIVE : This study was to compare the effect of silver diamine fluoride, casein phosphopeptide amorphous calcium phosphate (CPP-ACP), fluoride varnish on surface microhardness of enamel.

EXPERIMENTAL METHODS : 120 human premolar specimens were randomly divided into 4 groups as follows: 1. silver diamine fluoride, 2. CPP-ACP, 3. fluoride varnish, 4. control group (artificial saliva). Extracted premolars were cut mesio-distally with a slow speed diamond saw. One side of tooth specimens was randomly assigned. Each specimen was embedded in acrylic resin and the outer enamel was ground flat. Microhardness measurements at before and after treatment were performed using a Vicker microhardness test. After baseline microhardness was recorded, specimens were applied by each assigned material for 3 minutes and then stored in artificial saliva at 37°C for 6 hours. After specimens were subjected to microhardness testing. The median of the mean difference of the surface hardness was analyzed by Kruskal-Wallis test among the 4 groups and the surface hardness between two groups was compared by Mann-Whitney U test at a significance level of 0.05.

RESULTS : This study showed that silver diamine fluoride, CPP-ACP and fluoride varnish had significantly positive effect on enamel hardness ($p<0.001$). The no significant difference in hardness between silver diamine fluoride and fluoride varnish ($p=0.807$). However, there were significant differences between CPP-ACP and silver diamine fluoride ($p=0.017$), and between between CPP-ACP and fluoride varnish ($p=0.012$).

CONCLUSION : CPP-ACP showed the highest significant increase in surface microhardness of enamel followed by silver diamine fluoride, fluoride varnish and control groups.

D-10 (07-12) Translation, Validity and Reliability Testing of the E-San Short-form McGill Pain Questionnaire

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BACKGROUND : Short Form McGill Pain Questionnaire (SFMPQ) is a useful instrument for the assessment of pain. However it has not been translated into E-san language, which is the main language used in northeast Thailand.

OBJECTIVE : The objective of this study was to translate and test the validity and reliability of the E-san version of the SFMPQ (E-SFMPQ).

METHODS : This study was a descriptive study involving blinded and independent back to back translation of the English version of the SFMPQ by five expert individuals and subsequently tested for content validity. Pearson correlation coefficient, interclass correlation coefficient, cronbach's alpha coefficient were used to test the predictive validity, reliability and internal consistency of the E-SFMPQ respectively. Moreover, sensitivity and specificity of the E-SFMPQ were also assessed.

RESULTS : 186 orofacial pain patients were assessed by one interviewer and re-assessed again after 15 minutes with E-SFMPQ. Pearson correlation coefficient of musculoskeletal pain group and odontogenic pain group were high (>0.7) but neuropathic pain group was quite moderate (0.68). For reliability test of the total, sensory, affective, and evaluate total pain intensity, high intraclass correlations were demonstrated (>0.9 all group). Internal consistency was found to be high with Cronbach's alpha more than 0.8 for test and retest in musculoskeletal and odontogenic pain group. Comparing the sensitivity between the 3 groups, we found that the sensitivity of neuropathic pain and musculoskeletal pain were moderate but low sensitivity in odontogenic pain group. Specificity of neuropathic pain and odontogenic pain were high but low specificity in musculoskeletal pain. However, excluding the odontogenic pain group, the sensitivity of the neuropathic and musculoskeletal pain group was high at 0.8, and 0.9 respectively.

CONCLUSION : The E-SFMPQ developed is reliable, valid and cross-culturally equivalent to the original SF-MPQ questionnaire and responsive for the assessment of patients with neuropathic and musculoskeletal pains.

D-11 (08-1) Aesthetic Rehabilitation of Traumatized Maxillary Anteriors by Surgical Repositionings, Crown Restorations, Bleaching and Laminate Veneers

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Aesthetic rehabilitation of severely traumatized maxillary anteriors presents extreme challenges to the dentist. The prognosis of traumatized tooth depends on accurate diagnosis and physiological status of the involved tooth. Sometimes we should include adjacent tooth in treatment plan for aesthetic improvement. This case report describes aesthetic managements of traumatized maxillary anteriors using surgical repositioning and crown restorations, and of the adjacent teeth using vital tooth bleaching and laminate veneers. A 19-year-old female patient was presented with traumatized both upper central incisors. Clinical and radiographic examinations revealed complicated crown-root fractures with pulp exposure on both maxillary central incisors and intact both upper lateral incisors of peg-shape. After root canal treatment, surgical repositioning and crown restoration of the both upper central incisors, anterior aesthetic rehabilitation was completed by adding bleaching and laminate veneers on both maxillary lateral incisors. In the present case, even though the dentition possessed pre-existing aesthetic problems of peg-shaped adjacent teeth, more satisfying esthetic results could be obtained with special concern, such as bleaching and laminate veneers on the adjacent teeth following surgical repositioning and crown restoration of traumatized teeth. Therefore, it can be concluded that when we meet patients presenting traumatized teeth, a multidisciplinary approach will be necessary for the restoration of entire esthetics.

D-12 (09-4) Eagle's Syndrome Mimicking Orofacial Pain: A Case Report and Review of Literature

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Eagle's syndrome, which is associated with elongated styloid process, can lead to craniofacial and dental pain. It can also lead to recurrent pain at the oropharynx. It is often difficult to diagnose for the clinicians due its rare incidence and limited literature. Failure to give an accurate diagnosis will result in a loss of time and expenses and will cause undue stress to the patient and the clinician. This clinical report presents a patient with Eagle's syndrome who presented with painful headache, otalgia, and pain during swallowing. Its diagnosis and management through intra-oral approach is explained.

D-13 (09-5) Antimicrobial Effects of Grape Seed and Mangosteen Extracts against Microflora Associated with Percutaneous Skin Reactions Around Craniofacial Implants

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BACKGROUND : Severe skin reactions around craniofacial implants utilized for the retention of facial prosthesis have been noted to cause patient discomfort and can jeopardize treatment outcomes if left untreated. *Staphylococcus aureus* is one of the microorganisms associated with the skin reactions.

OBJECTIVE : To determine the antimicrobial effects of grape seed and mangosteen extracts against microflora associated with percutaneous skin reactions around craniofacial implants.

STUDY DESIGN : The extracts were initially screened for their antimicrobial properties against predominantly found microflora around craniofacial implants by disk diffusion test. The test specimens included reference strains of *Staphylococcus aureus (ATCC 6538)*, *Escherichia coli (ATCC 25922)*, and *Candida albicans (ATCC 10231)* and clinical strains of *Klebsiella pneumonia* and *Candida parapsilosis*. Modified agar dilution millipore method was used to determine minimum inhibitory concentrate (MIC) and minimum cidal concentration (MCC) of the extracts and to test the efficacy of a 50-50 admixture of polyethylene glycol and propylene glycol as a potential drug delivery media by combining it with various concentrations of the extracts. All tests were triplicated for confirmation.

RESULTS : Grape seed extract showed the most potent activity against *S. aureus* at MIC of 0.625 mg/ml and MCC of 1.25 mg/ml, whereas mangosteen extract showed inhibitory activity against *S. aureus* at MIC of 1.25 mg/ml and MCC of 2.5 mg/ml. On the contrary, both extracts showed low or no reactivity against *E. coli*, *K. pneumonia*, *C. albicans*, and *C. parapsilosis*. The admixture of polyethylene glycol and propylene glycol showed no antagonistic effects to the extracts and could be used as a potential drug delivery media.

CONCLUSION : Grape seed and mangosteen extracts along with their combination with polyethylene glycol and propylene glycol showed antimicrobial properties against *S. aureus*. These extracts can be studied further and used as natural alternatives to commercial drugs in the treatment of percutaneous skin reactions.

D-14 (09-6) Antimicrobial Activities of *Clinacanthus nutans (Burm.f) Lindau* Leaf Extracts against Oral Bacteria

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Clinacanthus nutans is commonly used as herbal medicines in Thailand. Previous studies have shown its anti-inflammatory effects and its promotion of the wound healing process. However, In Thailand, scientists found that dysentery and fever can be treated by this plant. Due to its anticell lysis property, the plant has been used as anti-venom for snake and scorpion bites and also removes nettle rashes. Therefore, the aim of this work is to study the antibacterial activities of *Clinacanthus nutans* extracts against *S. mutan strain KPSK2, E. faecalis ATCC 19433, and clinical isolate of L. casei.*

METHODS : The herb was extracted with 95% ethanol and was tested for antibacterial activities by agar disc diffusion method and and Minimum Inhibition Concentration (MIC) value against the bacteria.

RESULTS : The extracts of *Clinacanthus nutans* showed antibacterial activities against *E. faecalis* and *L. casei* strains in the range of 14.97-24.86 mm. *S. mutans* showed the most antibacterial activities (38 mm) in comparison to the control group; 0.2% Chlorhexidine mouthwash solution. The MIC value of *Clinacanthus nutans* extracts against *E. faecalis*, *L. casei*, and *S. mutans* were 15.63 µg/ml, 3.91 µg/ml and 0.49 µg/ml, respectively.

CONCLUSION : All bacteria tested were inhibited by the extract of *Clinacanthus nutans* with MIC that ranged from 0.976-31.25 µg/ml. The extracts from C. nutans were potential antimicrobial agents to control dental caries and periodontopathic bacteria.

Key words : Antibacterial activities, biological activities, *Clinacanthus nutans* extracts

D-15 (09-7) In vitro Study of Antibacterial Effects of *Curcuma Longa Linn* Extracts

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Curcuma Longa Linn, widely known as tumeric, is a rhizomatous herbaceous plant. It has long been used in traditional Asian medicines, it was used for treatment of viral, bacterial, and protozoan infection. Odontogenic infection causes a major dental problems. The odontogenic infection commonly occurred from both aerobic and anaerobic bacteria. Therefore, the oral bacteria associated with dental caries such as *Streptococcus mutan*, *Lactobacillus casei*, and *Enterococcus faecalis* were tested in this study.

OBJECTIVE : To study the antibacterial activities of the extracts against bacteria that associated with dental caries.

METHODS : Curcuma Longa Linn was performed by dried rhizome of turmeric at 40˚C for 3 days. The powder sieving system could be used for separating sized powder. The test organisms were *S. mutans* strain KPSK2, *E. faecalis* ATCC 19433, and clinical isolate of *L. casei*. All organisms were tested with different concentrations of the extracts. The MIC (minimtum inhibitory concentration) was determined using micro-broth dilution method.

RESULTS : The extracts showed antibacterial activities against *E. faecalis* and *L. casei* in the range of 15.03-33.00 mm. *S. mutans* showed the most antibacterial activities (38 mm) in comparison to the control group; 0.2% Chlorhexidine. The MIC of the extracts against and *S. mutans* was 0.976 µg/ml. On the other hand, the MIC of *L. casei* and *E. faecalis* were 7.82 and 31.25 µg/ml, respectively.

CONCLUSION : All bacteria tested were inhibited by the extracts with MIC that ranged from 0.976-31.25 µg/ml. The extract showed good Antibacterial effect against *S. mutans*. The extract could inhibit bacteria causing dental caries.

Key words : Antibacterial, biological activities, *Curcuma Longa Linn* extracts

D-16 (09-8) Antibacterial Activities of *Piper nigrum* Seed Extracts against Oral Bacteria

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Piper nigrum belonging to Piperaceae family is one of the most commonly used spices and is well-known as traditional medicine as well as its usage in the pharmaceutical and food industries. *Streptococci mutans* and *lactobacilli* are strong acid producers and hence cause an acidic environment creating the risk for cavities. *E. faecalis* has been frequently found in root canal treated teeth in prevalence values ranging from 30% to 90% of the cases. Root canal-treated teeth are about nine times more likely to harbor *E. faecalis* than cases of primary infections.

OBJECTIVE : To study the antibacterial activities of *Piper nigrum* seeds against *Streptococcus mutan*, *Lactobacillus casei*, and *Enterococcus faecalis*.

METHODS : The herb was tested for antibacterial activities by agar disc diffusion method and Minimum Inhibition Concentration (MIC) against *E. faecalis* ATCC 19433, and clinical isolate of *L. casei* and *S. mutan* strain KPSK2.

RESULTS : *Piper nigrum* seed extracts showed antibacterial activities against *E. faecalis*, *L. casei*, and *S. mutans* to inhibition zone 15.12, 24.99 and 34.39 mm, respectively. *S. mutans* showed the most antibacterial activities (34.39 mm) in comparison to the control group; 0.2% Chlorhexidine mouthwash solution 18.64 mm. The MIC of extracts of *Piper nigrum* seeds against *E. faecalis* and *S. mutans* was 31.25 µg/ml. On the other hand, the MIC of *L. casei* was 7.81 µg/ml.

CONCLUSION : All bacteria tested were inhibited by the *Piper nigrum* seed extracts with MIC concentration 7.81-31.25 µg/ml. *Piper nigrum* seed extracts could inhibit bacteria causing dental caries and periodontal diseases.

Key words : Antibacterial activities, biological activities, *Piper nigrum* seed extracts

D-17 (10-1) Application of 3D Modeling in Mandibular Tumor's Treatment: 2 Cases Report

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BACKGROUND : Recently, Three-Dimensional (3D) modeling has been particularly widely adopted in medical fields. Rapid prototyping biomodeling technologies using high resolution CT can apply to many aspects of Oral Maxillofacial Surgery. After osteotomies resection of mandibular tumour, bending of reconstruction plates is time-consuming and depends on the skill of surgeons. Bending plates on the 3D models prior to the surgery reduce operating times.

METHODS : A series of 2 patients with large tumors of the mandible underwent resection and simultaneous reconstruction with reconstruction plates. 2 patients were treated with 3D modeling and their reconstruction plates were bent prior to the surgery.

RESULT : Bending plates on the 3D models prior to the surgery significantly reduce operating times by saving time of repeated bending and adapting of plates. The mandibular symmetry achieved easily.

CONCLUSION : Using 3D modeling can accurately reproduce the morphology of an anatomic structure in oder to produce a real sized physical copy in polylactide (PLA), which is necessary to design treatment planning more exactly. 3D modeling defined the border of bone cutting line clearly, assisted surgeons in bending recontrustrution plates prior to the surgery to reduce operative time and improve esthetic quality.

D-18 (10-2) Soft and Hard Tissue Changes After Bimaxillary Surgery in Class III Patient

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BACKGROUND : In the modern society, the beauty is concerned by most people and more especially the face with harmonious shape and symmetry is very important if the patient had the malformation jaw. One of the most seriuos malformation of jaws is the class III. The proceess of treat this malfomation relative to bone and hard tissue. However, after sugery, the patient is not interested in hard tissue which change or not, they concern about the changing of soft tissue. Currently, in Vietnamese community, we has not found any researchs which focus about the changing of hard tissue and soft tissue, as well as correlations between changes in hard tissue-soft tissue after orthognathic sugery.

OBJECTIVES : The aims of this retrospective cephalometric study were to assess the results of bimaxillary surgery on subjects presenting with Class III patients and to evaluate the correlation between soft and hard tissue change.

MATERIAL AND METHOD : Soft and hard tissue changes were recorded by measurements of presurgical and postsurgical lateral cephalograms in acetate paper. 30 lateral; cephalograms (15patients) were selected which satified the sample selection. Pearson correlation coefficient were used to assess the degree of correlation in terms of soft to hard tissue changes between the two cephalograms.

RESULTS : The results showed that there was normalization of the cephalometric variables after surgery. Maxillary hard and soft tissue move forward. Otherwise, mandibular soft and hard tissue move backward. The changing of position showed a moderate correlation at two position Sn, A and Si, B in the horizontal direction.

CONCLUSION : The hard tissue was changed from Class III to Class I after surgery. The movement of soft tissue follow the changing position of hard tissue but this movement cannot be predicted.

D-19 (11-1) Analise the Ankilylosis in Temporo Mandibular Joint Using CBCT 3D (Case Study)

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BACKGROUND : Radiographic examination of the temporomandibular joint in the field of dentistry that is used as the investigation plays an important role in the diagnosis, determine the treatment plan, and evaluate the results of treatment. 3D CBCT tool able to demonstrate anatomical structures temporomandibular joint is clearly of the view coronal, sagittal and axial. The purpose of this study is to look at the ability of CBCT in interpreting abnormalities in the TMJ.

CASE : A patient came to the hospital dnegan complaints of pain and could not open his mouth. The doctor who examined suspected abnormalities in the TMJ and the patient is asked to do the CBCT.

RESULT : After CBCT where carried out at the open and closed positions, look for changes in shape and reduced flatness of articular eminantia position. Look also the conditions in which the distance between the fossa and condyle head attached. Physicians ensure that patients suffering from TMJ Ankylosis.

CONSLUTION : CBCT able to analyze abnormalities in the TMJ with good and accurate.

Key words : ankylosis, CBCT

D-20 (11-2) The Relationship between Level Vitamin D and Calcium Serum with Mandibular Bone Density in HIV/AIDS Childrens

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BACKGROUND : Human immunodeficiency virus (HIV) is a virus attacks the immune system of the body, ussualy was caused by HIV type 1. The proportion of women in new HIV infections in Indonesia has grown from 34 percent in 2008 to 44 percent in 2011 will lead a rise infections among children. There is an association between low vitamin D and HIV disease progression. Vitamin D is not only involve in calcium homeostasis which is have a negative impact on bone health, but also in regulation of immune system. Bone alteration have been observed in the course of HIV which reduced bone mineral density is the common bone lesion found in HIV patients. Bone mineral density is a parameter that predict fracture risk which in turn correlates with a shorter life expectancy. This research will study the relationship between level vitamin D and calcium serum with mandibular bone density in HIV/AIDS childrens.

METHOD : The research method is cross sectional study, serum 1,25-dihydroxyvitamin D and calcium levels were assessed from blood for randomly selected subject of HIV infected children enrolled treatment at Klinik Teratai FKUP Rumah Sakit Hasan Sadikin Bandung, West Java, Indonesia during March-June 2015. Panoramic radiograph were taken for measuring mandibular bone density.

RESULT : All 30 subject HIV/AIDS children showed serum 1,25-dihydroxyvitamin D were classified as vitamin D deficient (≤ 20nm/ml). A few subject showed an insuficient serum calcium level and all patient have low mandibular bone density.

CONCLUSION : Deficient vitamin D levels may lead lower mandibular bone density in HIV/AIDS children.

D-21 (11-3) Odontoma Cases Base on Cone Beam Computed Tomography (Case Report Study)

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OBJEKTIVE : To detect abnormalities in the oral cavity, particularly the swelling using radiography CBCT 3D.

CASE : Patient male Age 13 years, came to the Hospital of Faculty of Dentistry University of Padjadjaran with complaints gear 21 does not grow. Then do the photo to see 3D CBCT abnormalities impaction 21.

RESULT : The 3D CBCT radiographs found Odontoma on the impacted tooth 21. CBCT is not only used for the assessment of dental implant, but also various cases of disorders in the oral cavity, in this case the location and angulation of impacted teeth, cysts, abscesses, mesiodens, swelling and malignancy, making it easier for the surgeon to perform the operation.

CONCLUSION : CBCT can diagnose abnormalities odontoma.

Key words : Odontoma, CBCT

D-22 (11-4) Assesment of Antegonial Index of the Mandible on Panoramic Radiographs

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BACKGROUND : A number of measurements of jaw bone have been developed for use with panoramic radiographs. Antegonion index the best straight line was plotted along the anterior border of the ascending ramus and extended down to cross the lower border of mandible. The tangent to the lower border was drawn and a perpendicular to the tangent plotted, dotted line. Measurement of antegonion cortical thickness (antegonion index) was made along this perpendicular.

OBJECTIVE : The aim of this study was to assess Antegonial Index of the mandible on panoramic radiographs.

MATERIALS AND METHODS : This description study included 200digital panoramic radiographs of female and male patients that were grouped into 2 age groups (group 1: age 20-35; group 2: age 36-55). Index of the cortical width or thickness at the antegonion (Antegonial Index) were measured on both sides of the mandible in all panoramic radiographs.

RESULTS : The results of measured Antegonial Indexwas calculated, whereas in group 1 of female patients showed mean values 2,49 on the left side and 2,45 on the right side while in group 2 showed 2,73 on the left side and 2,55 on the right side.The index of male patients in group 1 had mean values 2,30 on the left side and 2,31 on the right side, while in group 2 showed 2,69 on the left side and 2,81 on the right side.

CONCLUSION : According to this study,male patients demonstrated lower mean value of Antegonial Index than female patients in age group 1. In addition, group 2 showed higher mean value than group 1 in male and female patients on both sides.

D-23 (11-5) Assessment the Shape of Tooth Base on Age Using Panoramic Radiographs of People in Bandung Indonesian

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OBJECTIVE : The aim of this study was to assessment the shape of the tooth base on age using radiograph panoramic in people, Bandung, Indonesia. Using the panoramic radiographs of people in Bandung Indonesia.

MATERIAL AND METHODS : 50 panoramic radiographs for each age group were observed. Cortical bone thickness was measured with mental index, base on Age in radiogaraphy on both sides of mandible. Average from all data for each group observed.

RESULT : Based on the survey results revealed that there were differences in the growth of every age ranging from ages 8-13, and can seemingly also that there are growth and development in line with in accordance with age.

CONCLUSION : The more we age; it also increased the growth and development of teeth.

Key words : Mental index (MI), Panoramic radiography

D-24 (11-6) Assesment of Gonial Index of the Mandible on Panoramic Radiographs

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BACKGROUND : A number of measurements of jaw bone have been developed for use with panoramic radiographs. The Gonial Index was introduced first and represents the thickness of the inferior mandibular cortex of the gonion region.

OBJECTIVE : The aim of this study was to assess Gonial Index of the mandible on panoramic radiographs.

MATERIALS AND METHODS : This description study included 200digital panoramic radiographs of female and male patients that were grouped into 2 age groups (group 1: age 20-35; group 2: age 36-55). Index of the cortical width or thickness at the gonion (Gonial Index) were measured on both sides of the mandible in all panoramic radiographs.

RESULTS : The results of measured Gonial Indexwas calculated, whereas in group 1 of female patients showed mean values 1,44 on the left side and 1,49 on the right side while in group 2 showed 1,28 on the left side and 1,55 on the right side.The index of male patients in group 1 had mean values 1,61 on the left side and 1,57 on the right side, while in group 2 showed 1,25 on the left side and 1,28 on the right side.

CONCLUSION : According to this study, male patients demonstrated higher mean value of Gonial Index than female patients in age group 1. In addition, group 2 showed lower mean value than group 1 in male patients on both sides while in female patients only on the left side.

D-25 (11-7) Healing Process in Patients with Osteoradionecrosis by Using Oxygen Hyperbaric Therapy (Case Study)

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BACK GROUND : Osteoradinecrosis is an infectious disease and inflammation that occurs in the jaw after doing radio therapy. Radiographic picture of the disease is visible lesions lucent extensive radio in the jaw. In Clinical condition, seen fistula and abscess in the jaw. One therapy for the treatment of this condition is the Hyperbaric Oxygen. The purpose of this paper is to look case report work hyperbaric oxygen therapy in treating osteoradionecrosis.

CASE : Patient age 55-year-old, woman, came with complaints of pain in left and right lower jaw, since one month ago, after doing radiotherapy for 6 months. Patients diagnosed osteoradionecrosis. For the healing, selected medical therapy and patients are advised for doing oxygen hyperbaric therapy in AL Minto Harjo hospital Jakarta.

RESULT : One month after doing hyperbaric oxygen therapies seen change and healing. Lesions that form still has the same broad but disappeared fistula, abscess dries, necrotic tissue disappeared/reduced as well as the formation of new bone matrix.

CONCLUSION : Oxygen therapy hyperbarik, obviously able to heal lesions caused osteomielitys osteoradionecrosis.

D-26 (11-8) Assessment of Mandible Cortical Bone with Mental Index Base on Age Using Panoramic Radiographs of people in Bandung Indonesian

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OBJECTIVE : The aim of this study was to assessment of mandibular cortical bone with mental index (MI), using the panoramic radiographs of people in Bandung Indonesia.

MATERIAL AND METHODS : 50 panoramic radiographs for each group were observed. Cortical bone thickness was measured with mental index base on Age in radiogaraphy on both sides of mandible. Average from all data for each group observed.

RESULT : There were significant different of mandible bone quality based on the tree technique, for each: group 5-11, 12-25, 26-45, 46-55 dan up 55 years old. The bone cortikal thickness rich on maximal in 26-45 years old then decline until this edge.

CONCLUSION : The bone cortikal using mental index with panoramic radiograph, shown diferent for each group.

Key words : Mental index (MI), Panoramic radiography

D-27 (11-9) Oral Lesions Related to Food Allergy

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BACKGROUND : The prevalence of allergies are increasing throughout the world in both developed and developing countries, including food allergies. According to World Allergy Organization, data in year 2013, as many as 240-550 million people may suffer from food allergies. The condition of food allergies can also occur in the oral mucosa, as oral allergy syndrome, recurrent aphthous stomatitis, and geographic tongue.

STUDY OBJECTIVE : Furthermore author intends to deliver a literature review regarding some food allergy-related oral lesions.

METHODS : Informations related to the topic were collected from scientific journals published on internet and also textbooks of Oral Medicine.

RESULTS : The characteristics of food allergies is the rapid onset following to the allergen and often belongs to Type I, IgE-mediated hypersensitivity. Some oral lesions due to food allergies, especially those found in adults such as Oral allergy syndrome (OAS) which is a class 2 food allergy, develops after aeroallergen sensibilization, as the allergens are mostly protein from plant origin (fresh fruits, vegetables, pollens, latex). Recurrent Aphthous stomatitis (RAS) also has been investigated to find out relation with some foods such as cow's milk, gluten, chocolate, nuts, or food additives. Other common oral lesion related to food allergy is geographic tongue (GT), a condition that has similarities in pathogenesis with rhinitis or asthma that both as a result of an acute inflammatory reaction. RAS dan GT has often been associated with atopy, which is a hypersensitivity condition to certain antigens include food allergens, characterized with exaggerated production of IgE, and have an inherited tendency.

CONCLUSION : The increasing incidence of food allergy in the world can lead to the emergence of oral lesions related to food allergy reactions, so as dentist should be able to recognize these lesions and perform proper treatment.

Key words : Oral lesion, food allergy

D-28 (11-10) Assesment of Dental Emerge Base on Age in Sundanes People Using Panoramic Radiograph

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BACKGROUND : Determination of the age, in some cases example disaster it is necessary, considering that Indonesia is a country vulnerable to natural disasters. Determination of the age can be made by several techniques which are based on the age of the existing teeth in the mouth. Research on the growth and development of the anterior teeth is sufficient a lot, as well as the molar teeth, but in contrast to the premolars. Research on the growth and development of the premolars are still rare. The aim of this research is to assesment of dental Emerge Base on Age in Sundanes People Using Panoramic Radiograph.

MATERIALS AND METHODS : This study used a method description with technical survey, conducted in the first and second premolars. The population is all of the data radiographs were aged 9-14 years. Data obtained by Emerge of the first and second premolars associated with age. The data is then processed and displayed in the form of tables and graphs.

RESULTS : Based on the results shown there are differences in dental Emerge different picture from every different age groups, and the increasing age, the dental emerge differently.

CONCLUSION : Growth and development of the first and second premolars using panoramic radiographs may help determine the age of a person.

Key words : Dental Emerge, age, growth and developmental of the teeth

D-29 (12-1) Load-deflection Characteristics of Bent Nickel Titanium Wire

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BACKGROUND : When leveling severe crowded teeth with nickel titanium (NiTi) wire, the more severity of displacement, the high force the wire exerts. Resulting in high chance of root resorption and periodontal destruction. Bent NiTi could produce the lower deactivation force with the less side effects.

OBJECTIVES : The study was designed to determine the natural characteristics of load-deflection rate of bent NiTi in different amount of bending and amount of deflection at 0.5 mm after deactivation.

METHODS : The three-bracket bend test was performed with an Intron universal testing machine (AMETEK Lloyd Instrument Ltd., Hamphshire, UK) in an environment of 37°C with the 10 N load cell and the crosshead speed of 1 mm/min. Two brackets were bonded on an acrylic block with 8 mm span and the central one was bonded on the acrylic crosshead.

0.012" bent superelastic NiTi (SuperElastic Regular Force, Highland Metals) with the placement of bends at 1 mm, 2 mm and 3 mm were tested at the same amount of total deflection at 1, 2, 3, 4 mm.

RESULTS : No hysteresis presented from 1,2 and 3 mm bent NiTi at 1 mm deflection. When amount of bends and the amount of deflections increased, the wires showed no springback characteristics. Bent NiTi with the placement of 1 and 2 mm bends produced lower forces.

CONCLUSION : To reduce the force of superelastic NiTi, to reach 50 to 60 g, bending of 1 and 2 mm in NiTi is recommended.

D-30 (15-1) Effect of Chitosan in Soft Tissue Healing Post Dental Extraction

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BACKGROUND : Chitosan is a deacetylated derivative of chitin, obtained from the hard outer skeleton of crustacean. In medical, it has been widely used for wound healing, skin grafts, haemodialysis and haemostasis. Whereas its utilization in post tooth extraction is limited.

OBJECTIVE : To evaluate the effectivity of chitosan on wound healing post simple dental extraction.

METHOD : Case control study on 20 subjects (male:female = 12:8) age 35-50, were divided equally into chitosan and control groups. All subjects were extracted 1 non-vital tooth. Post extraction clinical signs such as calor, rubor, tumor and dolor were recorded at day 2 and day 7. Chitosan gel was inserted into dental socket after tooth removal. No medication was given to all subjects. Wilcoxon Singed-Rank test was used for data analysis. Ethical clearance No.144/KE/FKG/10/2014.

RESULTS : There are significant difference between chitosan and control group on dolor and tumor signs on day 2 (P=0.001; P=0.03) and day 7 (P=0.012; P=0.029). Whereas significant difference on rubor sign was found only on day 7 between both groups (P=0.024). There is no difference between both groups on calor on both days.

CONCLUSIONS : Chitosan could suppress inflammation after dental extraction especially reducing pain, oedema and redness of the injury tissue.

Key words : chitosan, dental extraction

D-31 (15-2) Effectivity of *Andrographis paniculata* Mouthwash in Healing of Recurrent Aphtous Stomatitis Lesion (Preliminary Research)

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BACKGROUND : In dental practice, patients often complaint of recurrent oral ulceration. One of common oral ulceration is recurrent aphthous stomatitis (RAS). The prevalence of this condition is around 20% in population. *Andrographis paniculata* is a plant that widely used in the medical field because of several effects such as antihepatoxic, anti-malarial, anti-inflammatory, antioxidant, antimicrobial, anti-cancer, antiviral, antivenom, analgesic and antipyretic. In oral mucosa, these *A. paniculata* has not been evaluated. Even more, mouthwash medicament for oral lesion is limited to several products.

OBJECTIVE : to identify *A. paniculata* effect on recovery of RAS lesion compared to chlorhexidine gluconate and tetracycline as a mouthwash.

METHOD : this experimental research used double blind randomized clinical trial design approach. The subjects were 15 patients having episode of RAS from Trisakti Dental Hospital, Indonesia. Subjects were divided into 3 groups, *A. paniculata* 0.6%, chlorhexidine gluconate 0.1 %, and tetracycline 1.6%. Pain score was assessed everyday. Ulcer size was measured on day 1, 7 and 10. Ethical clearance No.156/KE/FKG/10/2014. Kruskal-Wallis test is used to analyze data.

RESULT : There is a difference found in reduction of pain score before and during therapy within each groups (*P*=0.066; *P*=0.043; *P*=0.039); but there was no difference in pain score before and during therapy between groups (*P*=0.255). There is no difference on duration of healing between all groups (*P*=0.527). Mean of duration of healing in *A. paniculata*, chlorhexidine gluconate and tetracycline groups are 4.8 days, 6.2 days, and 4.2 days. Based on the size of the lesion, there was no difference in lesion size reduction from pretreatment to 7 days treatment (*P*=0.291).

CONCLUSION : *A. paniculata* effect on the healing of RAS lesion similar to chlorhexidine gluconate and tetracycline.

Key words : Andrographis paniculata, aphtous stomatitis, healing.

D-32 (16-2) Affecting Factors of Bond Failure of Stainless Steel Brackets that Used at Public Health Center Kecamatan Jakarta-Indonesia

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BACKGROUND : There have been many research on the factors causing the bond failure (released of stainless steel brackets) in terms of its bond strength but there is no data that provide information about the factors that led to the released of stainless steel brackets in terms of patient.

OBJECTIVES : The objective of this study was to obtain data about the factors that affect the release of stainless steel orthodontic brackets in terms of the patient's diet, activities, the time and teeth's regio that stainless steel brackets often released.

EXPERIMENTAL METHODS : The study was conducted by means of a survey using a questionnaire containing questions regarding the factors that led to the released of stainless steel brackets in terms of patients' diet, activities, the time and teeth's regio that stainless steel brackets often released. Questionnaires (already qualified *ethical clearance*) were distributed at 10 of 44 (*Simple Random Sampling*) Public Health Center Kecamatan Jakarta-Indonesia in the period from April to June 2015. Twenty five subjects were taken from each health center so the total subjects were 250. Statistical Analysis of data is conducted by using Chi-square ($p < 0.005$).

RESULTS : Statistical analysis indicated that 240 patients had experience released stainless steel brackets, and significantly 150 patients experience released in the period 1-3 weeks after treatment. The teeth regio that stainless steel brackets often released are upper Premolar.The hard and sticky foods, activities such as sports, accidents and bad habits are also affect the released of stainless steel brackets.

CONCLUSION : The frequency of stainless steel brackets released is quite high mainly occurred in the period 1-3 weeks after treatment and the most released on upper Premolar. The diet and activities patient's are also affected the released of stainless steel brackets.

D-33 (17-1) Alteration of Mandibular Bone Density in type 2 Diabetes Mellitus Patients Based on Radiograph Examination

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Panoramic and periapical radiography serve as adjunct to establish the diagnosis of a disease. One of the features that can be observed in radiographs is the alteration of mandibular bone density. The determination of mandibular density is essential in diagnostic process, treatment planning and management of several treatment procedures such as implant placement, bone grafting and periodontal treatment. One of the systemic diseases which affect bone density is Diabetes mellitus (DM). Diabetes mellitus constitutes a group of common metabolic disorders that share the phenotype of hyperglycemia caused by impairment in insulin secretion, insulin action or both. The study aimed to determine the correlation of Diabetes mellitus by mandibular bone density. The decreasing of bone density in diabetic patients can be seen in radiograph. If the alteration of mandibular radiographic density in diabetic patients in relation to the duration of DM can be determined, it can serve as a guideline for dentists to manage DM patients. A total of 65 patients with a history of type 2 diabetes mellitus were taken from secondary data, (supplemented with interviews in the form of questionnaire). Periapical and panoramic radiograph examinations were made. To observe the mandibular bone density, the estimation was done by grading method. The results of mandibular bone density were correlated with age, hereditary, medication and duration of disease. Mandibular bone density was not significantly altered in type 2 diabetes mellitus patients ($p>0.05$). Similarly, mandibular density did not differ in patients with longer duration of DM type 2 ($p>0.05$), but the results showed that there was a relation between drug intake and mandibular bone damage ($p=0.006$). In conclusion was no alteration of mandibular bone density in type 2 DM patients according to age, duration of disease and familial history of DM, only drug intake history was significantly correlated with mandibular density change.

Key words : Diabetes mellitus, bone density, bone loss

D-34 (18-2) Oral Submucous Fibrosis —A Case Report of Young Patient with Stage II Oral Submucous Fibrosis

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Oral Submucous Fibrosis (OSMF) is a potentially malignant disorder having higher risk of developing cancer of oral cavity if untreated at early stages. The frequency of cancerous changes is reported to be 3% to 6% on OSMF cases. It is the most common precancerous lesion of the oral cavity in the South-East Asian, where the habit of betel quid chewing and its commercial preparations are high. Early diagnosis and prompt treatment is very important to prevent the lesion to be malignant. This is the case report of Oral Submucous Fibrosis occuring in 23 year old male with a history of betel quid chewing for five years duration. According to Pindborg JJ (1989), it is diagnosed clinically as stage II Oral Submucous Fibrosis. Scapel incisional biopsy was performed, the specimen was done for histopathological examination and immunohistochemical studying with Vimentin and Alpha Smooth Muscle Actin. Treatment with physiotherapy, nutritional support, intralesional steroid injection with quitting of areca nut chewing habit will be described in this case report.

D-35 (19-1) Collagen Triple Helix Repeat Containing-1 (CTHRC1) Expression in Oral Squamous Cell Carcinoma (OSCC): Prognostic Value and Clinico-pathological Implications

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BACKGROUND AND RATIONALE : Collagen Triple Helix Repeat Containing 1 (CTHRC1) is a 30kDa secreted and pro-migratory protein. This gene often found to be over-expressed in various types of human cancers. However, the correlation between CTHRC1 expression level with clinico-pathological parameters and prognosis in oral cancer remains unclear.

STUDY OBJECTIVES : Hence, this study aimed to determine mRNA and protein expression of CTHRC1 in oral squamous cell carcinoma (OSCC) and to evaluate the clinical and prognostic impact of this gene in OSCC.

DESIGN AND EXPERIMENTAL METHODS : In this study, mRNA and protein expression of CTHRC1 in OSCCs were determined by quantitative PCR (q-PCR) and immunohistochemistry (IHC), respectively. The association between CTHRC1 and clinico-pathological parameters were evaluated by univariate and multivariate binary logistic regression analyses. Correlation between CTHRC1 protein expressions with survival were analysed using Kaplan-Meier and Multivariate Cox regression models.

RESULTS : Current study demonstrated *CTHRC1* was significantly overexpressed at the mRNA level in OSCC (p = 0.001). Univariate analyses indicated high-expression of CTHRC1 was significantly associated with positive lymph node metastasis (p = 0.034), advanced stage pTNM staging (p = 0.01), and tumour size ≥ 4 cm (p = 0.011). However, only positive lymph node metastasis still remain significantly after adjusted with other confounder factors in multivariate logistic regression analyses (p = 0.044). Kaplan-Meier survival analyses and Cox model demonstrated that patients with high-expression of CTHRC1 protein were associated with poor prognosis and is an independent prognostic factor in OSCC.

CONCLUSION : This study indicated that *CTHRC1* expression was significantly over-expressed in OSCC compared to normal oral mucosa samples. The over-expression of CTHRC1 protein could be an independent predictor for nodal metastasis and low CTHRC1 expression level is a significant good prognostic marker for OSCC.

D-36 (20-1) The Relationship between Natural Head and Frankfort Planes in Mesh Diagrams Analysis

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OBJECTIVES : The aim of this study was to investigate the correlation between Na'Sn-natural head plane and Na'Sn-Frankfort plane in mesh diagrams analysis in Vietnamese adults, in order to find a interswitching method between these two angles from Frankfort plane in lateral cephalograms to natural head plane.

MATERIALS AND METHOD : This descriptive cross-sectional study was conducted on 68 lateral cephalometric radiographs (32 males and 36 females, aged between 18 to 25 years-old, evaluated by 10 orthodontists independently in 1999 as having harmonious appearance). Lateral cephalometric radiographs were taken in natural head position, traced, scanned with a 1:1 ratio, and measured with AutoCAD 2010 software.

RESULTS : Three characteristic angles (Na'Sn-natural head plane, Na'Sn-Frankfort plane and Pog''Pn-Frankfort plane) were selected to determine the relationship between natural head and Frankfort plane. A multi-variable regression equation estimating the correlation was established as follows: Na'Sn-natural head plane = 0.665×Na'Sn-Frankfort plane -0.347×Pog''Pn-Frankfort plane + 55.488.

CONCLUSION : The signihicant quantitative relationship between natural head and Frankfort planes through a regression equation can be efficiently utilized in mesh diagrams analysis among Vietnamese adults.

D-37 (22-1) A Patient with Facial Asymmetry and Unilateral Posterior Crossbite Treated with Surgery-first Approach

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BACKGROUND AND RATIONALE : Compared with conventional surgico-orthodontic approach, having surgery first eliminates the unsightly pre-surgical appearance and allows the chief complaint of the patient to be addressed at the beginning of treatment. In addition, immediate resolution of the soft tissue and skeletal imbalance is an added advantage in surgery first approach.

TREATMENT OBJECTIVE : In conventional pre-surgical orthodontics, the orthodontist tries to achieve a preoperative occlusion which is against what the soft tissue and skeletal components dictate. This has been thought of as one of the challenges in decompensating the arches prior to surgery. When surgery is completed first, the skeletal and soft tissue discrepancy is relieved and the teeth can be aligned without the need to fight with the physiological limitation.

Especially, In orthognathic surgery for correcting facial asymmetry, It is hard to decompensate teeth because of surrounding structures like tongue and cheek. Although dental decompensation is done, this structural imbalance could evoke trauma from occlusion or temporomandibular disorder.

In surgery first orthodontic treatment, structural balance and transient muscle atrophy induced from orthognathic surgery make it easier to decompensate teeth against occlusal forces or structural limitation.

TREATMENT DESIGN : To resolve the facial asymmetry, differential set-back of mandible body was planned. After BSSRO was performed first, postoperative orthodontic treatment was carried over. Transverse dental decompensation was done by posterior intrusion via mini-implants unilaterally.

TREATMENT RESULTS : The total period of active treatment was 18 months. Both patient's occlusion and facial appearance were significantly improved by the surgery-first approach.

CONCLUSION : We can definitely achieve treatment outcomes balanced with facial esthetics, functions, and stabilities through this surgery-first orthodontic treatment once a proper case-selection, diagnosis, and treatment planning are established.

D-38 (23-21) Changes of the Airway and Sleep-breathing Patterns of Skeletal II Children Undergoing Activator Therapy

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OBJECTIVE : Due to their similar mechanics by which both the Andresen Activator and the oral appliance for Obstructive Sleep Apnea Syndrome treatment advance the mandible forward, the aim of this study is to confirm if besides the intended inducement of development of the mandible the Activator may also help improving healthy sleep breathing patterns in children.

MATERIALS AND METHODS : Eight children aged 9 to 13 years currently undergoing activator therapy at Hiroshima University Hospital took part in this study. A portable sleep monitor was administered with the request of using it two different times, once wearing the activator and the second time without the appliance, indicators of severity included respiratory disturbance index, sleep fragmentation and cumulative time in respiratory effort, also obstructive, central and mixed events were considered; likewise two cephalometric radiographs were required following the previous requisites. Cephalometric measures included upper, middle and lower airway space width.

RESULTS : The results of these tests were analyzed and compared, from the sleep monitor tests it was found that sleep-breathing indicators of severity as well as all events were significantly decreased when the children slept with the activator in mouth rather than when not. From the cephalometric radiographs, it was found that when the appliance is in mouth there is a marked widening of the airways rather than when not wearing the activator.

CONCLUSION : The use of the Andresen activator not only induces proper development of the mandible but it also improves sleep breathing on children undergoing this kind of orthopedic therapy.

D-39 (23-22) Inhibitory Effects of Antibiofilm compound-1 on *Staphylococcus aureus* Biofilm

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BACKGROUND : A novel benzimidazole molecule, known as antibiofilm compound 1 (ABC-1) was found to prevent bacterial biofilm formation in various bacterial pathogens, without affecting the growth of bacteria (Sambanthamoorthy *et al*, 2011) while the targets of ABC-1 were unknown.

OBJECTIVE : We used *S. aureus* clinical isolates to study the effect of ABC-1 on biofilm components including surface proteins, Polysaccharide Intercellular Adhesin (PIA) and extracellular DNA (eDNA) during the biofilm formation process.

METHOD : 55 biofilm-forming clinical strains were selected from *S. aureus* collection of Department of Bacteriology, Hiroshima University. ABC-1 was synthesized in Department of Synthetic Organic Chemistry. The biofilm forming capacity was analyzed by microtiter plate assay. PIA production was detected by dot-blot using anti-PIA sera. Surface proteins were extracted by lysostaphin treatment in hypertonic condition and analyzed by SDS-PAGE. Expression of protein was analyzed by real-time PCR or by Western blotting. eDNA was extracted and quantitated by quantitative PCR using housekeeping genes.

RESULTS : Biofilm inhibition was observed in most of our strains upon treatment with 156 μM ABC-1. Dot blot analysis revealed that PIA production was reduced after the treatment of ABC-1. Real-time PCR and Western blot analysis indicated Protein A (SpA) expression was selectively down regulated. *spa* knockout mutant showed significant but not complete decrease of biofilm production suggesting involvement of other factors. ABC-1 treatment also decreased eDNA release, which was confirmed by qPCR.

CONCLUSION : Our results demonstrated that ABC-1 inhibited biofilm formation of *S. aureus* through affecting multiple components including SpA, PIA, and eDNA during early phases of biofilm formation without affecting the growth of bacteria. The detailed mechanism of multiple effects on biofilm components remains to be elucidated. However, ABC-1 could be a potential candidate as a new preventive agent to inhibit biofilm formation of *S. aureus*.

A-10 (07-13) PrepLooker: A Mobile Application for Assisting Dental Students in Learning Undercut & Convergence Angles of Crown Preparation

S. Aerarunchot, R. Chotipanvidhayakul, N. Boonpongsathian, T. Songphaeng, K.R. Saikaew and P. Buayai

ABSTRACT : The undercut and convergence angle are the factors affecting retention and resistance to the crown, but the accuracy of its visual estimation has not been determined. It is difficult for the inexperienced dental students to evaluate the undercut and convergence angle, so they might not place the abutments for porcelain fused to metal crown properly. The objective of this study was to evaluate the effectiveness of a mobile application, PrepLooker, in learning about undercut and convergence angles. This study conducted an experiment with twenty of the 6th year dental students who did the tests before using the application. The tests were the ten artificial teeth prepared to be the abutments for porcelain fused to metal crown, with convergence angle ranging between 0-20 degrees and more than 20 degrees and with and without undercut. All of the abutment teeth were checked for the validity and reliability by three experts. After that, the dental students studied how to determine and estimate undercut and convergence by using the application for one hour. Then the 6th year dental students did the post-test without using the application. The average scores before and after using application were compared with Paired T-test (P-value < 0.025). This study result showed that the application can assist the 6th year dental students to understand undercut & convergence angles of the crown preparation. The average scores after using the application were increased significantly by 4.5%. In the future, the application would be developed for actual usage in clinical situations.

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