4th Hiroshima Conrference on Education and Science in Dentistry

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PREFACE


HIROSHIMA CONFERENCE ON EDUCATION AND SCIENCE IN DENTISTRY *

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Research activities of Hiroshima University: Now and future

E. Tsuchiya

Excecutive and Vice-President (Research), Hiroshima University

Over the past decade, Japanese universities have undergone radical environmental changes. Especially, the trend of deregulation accelerated after the incorporation of national universities, resulting in intensified inter-university competition. In this environment, individual universities are expected to establish new frameworks and systems for their respective institutions. Hiroshima University is also expected to establish its own system, based on a concrete future vision.

Hiroshima University is recognized as one of the key universities in Japan. In 2009, the Long-Term Vision of the University has been prepared and presented the direction of Hiroshima-University's future development as a "Comprehensive Research University" and a National & Regional Academic Center. In addition, the University will determine the roles unique to Hiroshima University as a regional hub of education, research, medical services and community development programs. Moreover, we will work to expand the reach of our activities, particularly those in the University's strong disciplines, across the nation and the world. Especially, in addition to the development of excellent human resources, the main mission of higher education institutions, our university conducts research from the basic sciences to the distinctive and advanced in a wide variety of fields as a world-class educational and research center. By providing advanced specialized knowledge gained from such research activities to its faculties and graduate schools, we seek to develop human resources who can lead various fields with their knowledge and skills, thereby greatly contributing to the construction of the future of society. Here, I will introduce some cutting-edge researches published in this year, and discuss how to achieve the mission of Hiroshima University in research.

CUTTING-EDGE RESEARCH PROJECTS IN RECENT YEARS

Engineering:
Developing a nano-optical antenna

Prof. Y. Kadoya (Grad. S. Advanced Sciences of Matter) *et al*. developed a nano-optical antenna that can detect visible light. This miniscule antenna is one-millionth the size of the Yagi-Uda antenna invented in Japan 80 years ago and has been used worldwide to receive radio-waves for television broadcast. Achieving both enhanced emission and detection of light-waves, their device is expected to enable effective optical molecular analysis as well as optical interconnections between molecules, potentially contributing to the advancement of future communications technology. It is expected that the antenna improves the performance of single photon sources that can be used for quantum computing and communication.

Barrier-free travel environment with an original software in Japan

Prof. A. Fujiwara (Grad. S. International Development and Cooperation) *et al*. developed an application for smartphones to help the hearing-disabled use public transportation. This is an original application in Japan designed specifically to aid the disabled in travel. They concentrated on usability from the disabled individual's perspective in development and took advantage of the higher potential and flexibility of smartphones. Intended primarily for the hearing-disabled who wish to travel by bus, the purpose of the application is to alleviate the anxiety of being unable to hear on-board announcements by providing useful information to users via the display such as notification of approaching their destination. We conducted a monitoring survey at a tourist spot. The disabled monitors rated the application highly, saying it was extremely helpful in understanding their current location and obtaining fare information on board. We hope our research will contribute to the advancement of a society free of physical and mental barriers that hinder travel.

Life Science:
Artificial swine insemination using frozen semen: Creating a next-generation pork production system that benefits the Japanese pork industry

As. Prof. M. Shimada (Grad. S. Biosphere Science) *et al*. developed a freezing process to preserve swine semen as well as a method for artificial insemination utilizing this frozen semen. Their results in superior reproduction performance compared to existing techniques. By semi-permanently preserving semen in liquid nitrogen, this technology allows the conservation of valuable purebred swine genomes, for example, during outbreaks of foot-and-mouth disease and other infectious epidemics. Because of its low cost, relative convenience, reproduction performance and high level of safety, it is believed that the technology can significantly benefit the Japanese pork industry by providing a more efficient and stable production system.

Medical Science:
Identifying the gene responsible for amyotrophic lateral sclerosis: Discovering mutations through recessive inheritance

Prof. H. Kawakami (Research Institute for Radiation Biology and Medicine) *et al*. discovered a gene that is involved in the onset of amyotrophic lateral sclerosis

(ALS). Until now, the mechanism of onset was unknown. Focusing on autosomal recessive inheritance in pedigrees, they analyzed high-density single-nucleotide polymorphisms (SNP) to narrow down candidate regions, detected a mutation of the Optineurin (OPTN) gene, and proceeded to investigate how the gene is involved in the mechanisms of onset. We hope to fully understand this mechanism in the future in order to develop a treatment for the disease.

The World's first successful development of human autism in a mouse model

Prof. T. Takumi (Grad. S. Biomedical Sciences) succeeded in developing the world's first model mouse with a chromosomal abnormality similar to that of human autism. For this research achievement he was awarded second prize in the 46th (2009) Baelz. Behind this research are the remaining unknown factors of psychiatric diseases, as opposed to the ongoing molecular-level elucidation of various physical diseases that has taken place since the completion of the human genome sequencing. Like many other diseases, autism is caused by a biological abnormality. Accordingly, Prof. Takumi attempts to clarify the mysteries of human mental disorders by using embryological engineering techniques that enable him to produce and genetically examine model mice with human psychiatric diseases, to explore and analyze genes that may be responsible for abnormal psychiatric behavior.

Education:
Learning about today's Japanese from the history of the 'on' pronunciation of kanji (Chinese ideograms) — "on" is the pronunciation from a foreign language

Prof. I. Sasaki (Grad. S. Education) conducts research into the past use of kanji. Kanji are originally foreign characters pronounced with foreign sounds, and are now pronounced in on and kun ways in Japanese. They are essential in writing Japanese. Prof. Sasaki discovered that words written in kanji in the past were pronounced differently. They had different accents depending on each speaker's proficiency, the purpose behind the use of the words and the social context of their use. This is like English words in Japan today. Prof. Sasaki compiled the results of his research into "Heian-Kamakura-jidai niokeru Nippon Kan'on no Kenkyu (Research into the Han-reading of Chinese characters during the Heian and Kamakura eras)" and was awarded the Shinmura Izuru Prize in 2009 (presented to those making an important contribution to Japanese philology or linguistics). Recently, Prof. Sasaki focuses on the use of kanji by one person, Shinran, who is a great Buddhist figure. Prof. Sasaki is trying to decipher the pronunciation of all kanji used in the hand-written documents left by Shinran in order to clarify the phase contrast of sounds in the Kamakura era.

WINNERS OF "FUNDING PROGRAM FOR NEXT GENERATION WORLD-LEADING RESEARCHERS"

The Funding Program for Next Generation World-Leading Researchers (NEXT Program) is implemented under the Leading-edge Research Promotion Fund established within the Japan Society for the Promotion of Science (JSPS) using the Japanese government's FY 2009 supplemental budget. This program provides a research-support system for researchers expected to have the potential to be world leaders in their respective fields of science and technology. The following four researchers of Hiroshima University won the project. We are expecting their great success.

Ken-ichi SAITOW, Professor, Natural Science Center for Basic Research and Development, Fabrication of nanoSi-whitelight-emitting device and nanoSi-solar cell

Seiichiro HIGASHI, Professor, Graduate School of Advanced Sciences of Matter
Single crystalline growth of semiconductor using ultra-high-density atmospheric pressure plasma jet and its application to large-area electronic device fabrication

Hirofumi MARUYAMA, Associate Professor, Research Institute for Radiation Biology and Medicine, Research on mechanisms of optineurin and related subjects in amyotrophic lateral sclerosis.

Takashi KANEMATSU, Professor, Graduate School of Biomedical Sciences
Researches on functions of a new protein regulating energy metabolism and food intake

Special Lecture

Special Lecture I

Personalized medicine: Is dentistry ready?

National Institute of Dental & Craniofacial Research

R. Kuska, I. Garcia and M.J. Somerman

Special Lecture II

Innate defense peptides in the oral cavity

The Forsyth Institute, Department of Immunology

T. Kawai

Personalized medicine: Is dentistry ready?

R. Kuska[1], **I. Garcia**[1] **and M.J. Somerman**[1,2]

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Key words: Personalized medicine, genomics, diagnostics, pharmacogenetics, dentistry, oral diseases

ABSTRACT

Personalized medicine involves tailoring of care to meet a patient's individual needs. While dentists and physicians long have personalized care based on their patients' needs, environment and history, in recent decades the concept has expanded to incorporate knowledge about the patient's unique biology and genetic makeup. This article envisions the likely impact of a personalized medicine revolution on dentistry by addressing four areas: 1) building the personalized revolution, 2) biology-based dentistry, 3) dentistry's expanded healthcare role, and 4) the current barriers to progress. Biology-based care has the potential to transform the profession of dentistry and expand the role of dentists as part of the primary care network. Our profession is well positioned to take advantage of the expansion in understanding of molecular profiling, metabolomic analysis, enhanced diagnostics and genetic testing. But as genetic testing becomes more common, it is clear dentists will need further education and training in genetics; they also will need to keep up with rapidly changing technologies in order to understand the complexities and limitations of such approaches to clinical care. Our profession must come together now and engage in dialogue focused on preparing the current and next generation of practitioners, as well as the public to fully understand and thus adopt personalized medicine as part of our means to provide the best quality of care for the communities we serve.

BACKGROUND

Biomedical research is communicated in a highly specialized language that can be difficult for many to navigate. But terms sometimes emerge that speak to all. Personalized medicine is a prime example. In its most general sense, personalized medicine describes the tailoring of care to meet a patient's individual needs. This, of course, is nothing new. Dentists and physicians long have tailored care based on a patient's environment, behavior, and health and family history (Fackler et al., 2009). In the 1970s and 1980s, however, personalized care expanded conceptually to include targeted therapeutics, a then investigational melding of clinical research and basic biology, particularly basic cancer research. For example the recognition that certain cancer cells could be selectively targeted based on the type of receptors expressed allowed for more directed therapies. The hope was rationally designed treatments would serve as "molecular medicine" that restored the defective cellular signaling pathway (s) causing a patient's disease. These first-generation smart drugs were hailed as potentially more effective, efficient, and likely to elicit fewer side effects than existing non-specific, saturate-and-heal treatments.

In the 1990s, the plot and definition of personalized medicine thickened with the first wave of predictive genetic tests, an outgrowth of incremental progress in the positional cloning of disease-linked genes (Vnencak-Jones, 1999; Kuska, 1996). Then, in the 2000s, a breathtaking run of informational and technological progress in genetics spawned the new field of personalized genomics. It bets that the technologically sophisticated study of the whole genome will solve the mystery of its complex structure and ultimately crack the molecular foundations of individual genetic variation.

With this cracking sound, a historic paradigm shift would ensue. Today's Oslerian tradition, born in the late 19th century to define disease, based on the organ system in which signs and symptoms manifest (usually after the diagnostic fact), would give way. Replacing it would be a molecular-based classification system of human disease. The new system would provide the diagnostic rationale for clinicians to peer into a suspicious cell and look for early disease-spawning breakdowns in the genome or its myriad signaling pathways. With this information, practitioners would no longer lump patients under the same semantic heading of, for instance, oral cancer. They would have a molecular printout in hand on a patient's tumor. The profile would characterize its biochemical nature and help practitioners steer the patient "to the right drug at the right dose at the right time" (Hamburg et al., 2010).

In the early 2010s, personalized medicine remains a conceptual mix of something old, something new (Steele, 2009). But the new already has left its first tangible imprints on patient care. Examples include: The CYP2C9/VKORC1 gene test that helps physicians prescribe a subset of their patients with the right dosage of the blood-thinner Coumadin® (Moyer et al., 2009);

Herceptin®, a monoclonal antibody that flags which breast cancers over express the *Her2/neu* receptor and thus helps oncologists make the right chemotherapy choice (Tokunaga et al., 2006); and the AmpliChip CYP450 genotype test to determine which patients are poor metabolizers of about a dozen drugs, guiding them to the right medications (De Leon et al., 2006).

These tangible imprints likely will grow more pronounced in the years ahead. The public and private sectors continue to make significant investments in personalized care. According to a 2010 report from the Tufts Center for the Study of Drug Development, up to 50 percent of surveyed companies have personalized medicines under development as potential therapies for a range of indications including cancer, cardiovascular disease, and immunological conditions (Milne, 2010).

The public and private sectors also continue to invest in the needed infrastructure to help scientists better traverse the uncertain terrains of genomics and targeted therapeutics. The U.S. federal National Institutes of Health (NIH), for example, supports big-science initiatives such as the Encyclopedia of DNA Elements (ENCODE) project, which aims to identify all functional elements in the human genome; the NIH Roadmap Epigenomics Program that seeks to define a parts list of the human genome; the Cancer Genome Atlas, which maps the genetic changes in 20 cancers; and the Therapeutics for Rare and Neglected Diseases (TRND), to enable promising compounds, including targeted therapies, to be taken into the preclinical development phase of the drug development process.

This article envisions the likely impact of a personalized medicine revolution on dentistry. It does so by addressing four points: 1) building the personalized revolution, 2) biology-based dentistry, 3) dentistry's expanded healthcare role, and 4) the current barriers to progress.

Building the Personalized Revolution

First and foremost, building a revolution in personalized care stands as the tallest of biological tasks. Humans consist of 210 cell types, approximately 35,000 genes, an estimated 10 million proteins, and as many as 3,000 metabolites. In addition, human cells contain 95 trillion water molecules, 60 billion proteins, 2 trillion fat molecules, 5 trillion sugars and amino acids, 60 billion RNA molecules, and six feet of DNA (Naylor et al., 2010). All of these variables in theory must be present and accounted for in the difficult computational task ahead of linking genotype to reliable phenotype to disease state.

So must the inherent complexity of cells as integrated, information-processing systems. Take the human genome itself. In the decade since the completion of the Human Genome Project, a standard working theme is genes and genomes are more complex structurally and functionally than previously appreciated. Venter notes that the first fully sequenced human genomes turned up a surprising. 01 rate of variation from person to person. Technical refinements and multiple re-sequencing of the human genome have bumped up the variability rate from one to three percent (Venter, 2010). Likewise, the NIH-supported ENCODE Pilot Project (2004-2007) reported numerous surprises in its fine-tooth-comb analysis of one percent of the human genome. The surprises included complex transcript networks, extensive transcript overlaps, phantom transcription start-sites, gene regulation in cis governed millions of bases away, and, most surprisingly, 60 percent of human DNA may be under evolutionary constraint, that is, resistant to evolutionary change (Encode Project Consortium, 2007). All were previously unknown or poorly appreciated.

There is, however, reason for encouragement. Sequencing costs continue to plummet. As of January 2011, the human genome can be sequenced for approximately $11,000, (http://www.genome.gov, 2011) and the Holy Grail-price tag of a $1,000 or less appears to be well within reach. The lower costs, coupled with vastly increased sequencing speeds, will make feasible in the not-so-distant future the mass sequencing of tens of thousands of human genomes. The more copies of the human sequence to examine, the greater the likelihood to find interesting sequence variations and new information on epigenetic phenomena, such DNA methylation, and their roles in the non-Mendelian imprinting of traits.

Another reason for encouragement is the revolution will be multidisciplinary, i.e., the whole stands to be greater than the individual parts. Genomic scientists work in close alliance with their colleagues in a number of newly minted, so-called 'omics subspecialties. These include transcriptomics, which helps to collect vast stores of data on gene expression; proteomics, which profiles the variable patterns of protein expression in our cells; and metabolomics, which catalogues the small molecule byproducts, or metabolites, from chemical reactions throughout our bodies (Personalized Medicine Coalition, 2009). Systems biology, the study of cellular networks, also plays a leading role. This relatively new discipline will prove invaluable in the mathematical modeling of cells as integrated, information-processing systems.

How will this digitized critical mass of biological information one day yield a new molecular classification system of disease? The next – and critical – point will be to link well-defined genotypes to reliable clinical phenotypes. In other words, reveal the still mostly hidden relationships between genetic variation and biological outcomes, namely, human health and disease (Venter, 2010). To uncover them, extreme rigor will be required at the front end of the investigative process to define unequivocally genotype and phenotype. At the backend, the imperative will be to maximize computational power and to find the "few needle variations in the haystack of complex information" (Hoffmann et al., 2011)

Assuming the needles fall out, more computational power will be required to integrate and assemble the data to define the many distinctive molecular signatures of a given disease state. These subtypes could, and likely will, number in the tens or hundreds, much like the entry in an American telephone book for the name Smith. Drug developers, in turn, will dial up each unique molecular signature and design medicines to target them directly. This so-called pharmacogenomic approach will personalize therapeutics on a broad scale.

In the language of baseball, personalized medicine will hit many more singles and doubles before loading up to attempt the home runs listed above. But, as Collins

rightly advises, "Those who somehow expected dramatic results overnight may be disappointed, but should remember that genomics obeys the First Law of Technology: we invariably overestimate the short-term impacts of new technologies and underestimate their longer-term effects" (Collins, 2010).

Biology-based Dentistry

How does this research story relate to the everyday practice of dentistry? Let's take a look, starting with oral pathology. Few would disagree that oral diseases, such as periodontal disease, oral cancer, and the dry mouth associated with Sjogren's syndrome, are complex disorders. They involve a complicated mix of genetic, biological, behavioral, and environmental factors. They also can be highly variable among individuals and populations.

What about dental caries? Although caries tends to fall under the rubric of preventive and restorative dentistry, a genetic component clearly contributes to the problem (Wang et al., 2010; Borass et al., 1988). For example, scientists recently scanned the genomes of multiple families in the American states of West Virginia and Pennsylvania in search of sequence variants that might show an association with increased risk for dental caries or, conversely, protect against the disease. They found two genes, TAS2R38 and TAS1R2 which were statistically significant. Both are involved in mediating the sensation of taste (Wendell et al., 2010). Although the data are preliminary, the implication is some people may have a gustatory predisposition to eat cariogenic foods – and vice versa. Thus, this finding potentially brings personalized genomics into the dentist's office as a consideration in the optimal practice of preventive dentistry.

And so the story will go in the months and years ahead. With low-cost sequencing and high-powered computation churning out new leads, oral diseases soon will reveal more of their biological secrets than ever before. It is safe to say, the more revealing the biology that filters into everyday dentistry, the more practitioners will find themselves out of necessity, adding to their familiar hand-held devices to treat disease, high-speed molecular diagnostic technologies, many that will be hand-held, that profoundly inform their treatment decisions and customize them to their patients' needs.

Adding to the biological drumbeat will be the tremendous ongoing progress in other areas of dental science. These include microbiology, immunology, tissue engineering, imaging, neuropharmacology, stem cell biology and nanotechnology.

Taking these disciplines and a speculative glance further down the road, a common thread sticks out. Biology-based dentistry will transform the most fundamental principle of the profession: restoration of form and function. No longer will dentists rely as readily on traditional radiographs, mechanical instruments, and ceramo-metallic materials to visualize and repair damaged tissue. They will regenerate form and function (a) using the precision of molecular information as their operational guide to customize treatment and (b) employing the body's own cells and biochemistry as their engineering materials.

Future practitioners will have a range of targeted therapies at their disposal to turn off the immune response in the mouth and better control chronic inflammation of the tooth-supporting periodontium. They also will no longer manually scale and plane the tooth root as a primary means to treat those with early periodontal disease. Moreover, current surgical procedures used to treat advanced infections will be replaced by more biological host-tissue specific targeted approaches. Dentists will have the option to insert a tiny plastic scaffold into the wound. The three-dimensional scaffold, measuring a few millimeters in diameter at most, will degrade within minutes or hours and release a natural work force of stem cells and other biologics to regenerate the damaged tissue and restore its function anew.

Future dentists also will have the diagnostic tools to access a broad spectrum of molecular information inside the mouth. This information will indicate which oral pathogens are predominant in the dental biofilm of a patient with chronic periodontitis, allowing treatment to be tailored to their molecular characteristics. Or, they will profile in their offices the circuitry of a tumor cell biopsied from the tongue. The diagnostic work up will guide the choice of chemotherapy drugs to those on the shelf that are most likely to target the internal wiring of the tumor cell and kill it. Or they will detect demineralization of hard tissues at stages that allow remineralization versus more aggressive therapies to remove caries and will better identify individuals at risk for caries at an early age. In short, no longer will dentists saturate damaged tissue with non-specific drugs or anti-bacterial treatments that are often not effective and/or not predictable and often destroy the good with the bad. They will target their treatments to remove the bad and leave the good untouched.

Dentistry's Expanded Role in Healthcare

Another common thread will be the expanded role of dentistry in healthcare. In the United States, the change will be catalyzed in part by the national mandate to adopt a unified electronic medical recordkeeping system in 2014. This shift allows health professionals to speak a common language and opens the door for dentists to join their primary care colleagues in a more team-oriented, multidisciplinary network of healthcare delivery.

Within this paradigm, dentists have the potential to serve as gatekeepers within the healthcare system. This owes to three factors. One, dentists traditionally see most of their patients twice a year for routine checkups and preventive care. Two, the mouth is easily accessible and, indeed, the gateway to most of the body. Three, in the coming era of personalized care, dentists will provide a diagnostic tool set, which will include cancer, caries, mineralized tissue, and salivary diagnostic screening during these visits to monitor for telltale molecular signs of developing oral and systemic diseases as well detection of existing oral/systemic pathologies.

Saliva has several advantages over blood as a diagnostic fluid. These include easy, noninvasive collection, potential for lower costs associated with testing, easy portability, and point-of-care application (Miller et al., 2010; Malamud, 1992). Over the past decade, the

National Institute of Dental and Craniofacial Research has supported research initiatives to better anchor the scientific foundations of salivary diagnostics and broaden its technological capabilities. Scientists already have established in saliva the presence of disease-related proteins for several cancers, cardiovascular disease, connective tissue disorders, periodontal disease, and other conditions (Miller et al., 2010).

As now envisioned, upon entering a dentist's office, patients will spit into a small plastic tube. Staff will walk into a nearby room and load the contents into a disposable diagnostic chip about the size of a dime. This all-in-one chip will scan the saliva to determine the levels of numerous molecules in the fluid and produce a detailed print out within minutes. Staff then will compare unusual decreases or elevations in various proteins, antibodies, or other analytes with the patient's last checkup. At the end of the visit, the dentist will review the results with the patient. If the profile shows something unusual, the dentist will advise the patient on how to follow up within the healthcare system to obtain a more definitive diagnosis. Additional diagnostic tools are being developed to improve on our ability to detect disease at early stages. One example is the application of nano-bio-chip sensors for analysis of oral cancer biomarkers that show higher sensitivity and specificity than those currently available (Weigum et al., 2010).

As these examples indicate, as personalized medicine advances and its biological commonality in all parts of the body is better appreciated, the artificial divide between dentistry and medicine will narrow. Dentists increasingly will join their medical colleagues in a more coordinated, multidisciplinary network of healthcare delivery.

The Current Barriers to Progress

Collins and Hamburg recently likened the pursuit of personalized medicine to the construction of the national highways system in the United States after World War II (Hamburg et al., 2010). In the latter case, the federal government built the interstate highway system, and motorists were free to drive wherever they wanted without interference. The same holds true for personalized medicine. The needed investigative infrastructure is largely in place, and scientists are free to travel wherever their research interests take them to pursue a future of personalized medicine.

Where will this scientific infrastructure take practicing dentists? Like desktop computers and the emergence of internet in the 1990s, some dentists will be early adaptors to biology-based dentistry. Others will be more cautious in their approach, yet it seems evident that dentistry will only benefit from the emergence of these biological tools and their ability to better tailor care to patients. The profession may want to start from the premise that personalized healthcare looms on the horizon. True, Rome wasn't built in a day. But the fruits of this investment won't mature all at once. Early advances could potentially benefit dentistry within years, and early preparation to identify possible barriers to their smooth implementation can make change less jolting.

More than ever, dental students will need to be versed in biology and the scientific underpinnings of personalized care. But as genetic testing becomes more common – it is unclear how well prepared health care providers, including dentists will be to interpret them. Clearly, dentists will need further education and training in genetics; they also will need to keep up with rapidly changing technologies – including genomics and the whole 'omics' field – to be able to understand the complexities and limitations of such approaches to clinical care. And as patients become more educated and empowered to understand the benefits and possible risks of personalized medicine, dentists will find themselves in uncharted waters as patients may approach them and ask for their guidance.

CONCLUSION

Over the past decade we have experienced marked advances in technologies used to diagnose and to treat diseases associated with the dental-oral-craniofacial complex, resulting in more-evidence and biologically-based approaches for taking care of our patients' health care needs. With these advances the need to focus our attention on more multidisciplinary tactics to provide the best care for our patients are emerging, albeit slower than many of us would like. Examples of multidisciplinary practices include the increase in interdisciplinary courses and clinic training offered by our professional schools and the increase interactions between pediatricians and pediatric dentists, including delivery of fluoride by physicians and/or enhanced bidirectional referrals (Close et al., 2011; Assoc. of American Medical Colleges, 2008; JDE Supplement, 2008).

Thus our profession is well positioned to take advantage of the exponential expansion in our understanding of molecular profiling, metabolomic analysis, enhanced diagnostics and genetic testing to provide personalized health care approaches for prevention, diagnoses and treatment of our communities.

We can foresee a day when no patient will experience intolerable pain, loss of function, or disfigurement from late-stage oral diseases. Instead oral health professionals will use information about the patient's genetic profile to offer a more precise diagnosis, tailor his or her care, and preempt disease before it manifests clinically.

But the challenges/hurdles that must be dealt with for this to happen are significant (Table 1). The current climate in our nation, with states and the federal government facing marked budget deficits, has created considerable tension around health care policies and worsened access to care. Health care expenses have escalated, while the communities not able to afford health care have increased. Moreover personalized medicine adds other concerns around scientific, educational and socio-ethical and legal barriers that will need to be overcome for genomic medicine, stem cell therapy and other molecular approaches to care to be recognized as cost-effective methods for saving lives and improving the quality of health for all individuals. Our profession must come together now and engage in dialogue focused on preparing our next generation of practitioners and leaders, our students, our current practitioners, as well as the public to fully understand and thus adopt personalized medi-

Table 1. Personalized Medicine: Advantages Envisioned & Hurdles to Overcome

Advantages:
• Early diagnosis and intervention before diseases manifest clinically
• Decline in disfigurement of the mouth, head and face
• Narrow the inequality gap in oral health between population groups
• Increase in the cost-savings of oral health care
• Improve quality of life and well-being of patients
• Expand opportunities for dentistry to be part of multidisciplinary health care
Hurdles:
• Evidence of improved outcomes
• Evidence of decreased expense long-term
• Social, Ethical and legal barriers
• Expertise by clinicians required for a personalized medicine approach
• Resistance to change and/or to acceptance of new procedures
• Curriculum modification at the educational/professional schools
• Communication and messaging for the public

cine as part of our means to provide the best quality of care for the communities we serve.

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REFERENCES

1. Assoc. of American Medical Colleges. (2008). Report IX Contemporary Issues in Medicine: Oral Health Education for Medical and Dental Students. Accessed 7/22/2011 at www.aamc.org
2. Borass JC et al. (1988). A genetic contribution to dental caries, occlusion, and morphology as demonstrated by twins reared apart. *J Dent Res* 67: 1150-1155.
3. Close K, Rozier RG, Zeldin LP, Gilbert AR. (2010). Barriers to the adoption and implementation of preventative dental services. *Pediatrics* Vol. 125 No. 3.
4. Collins FC. (2010). Has the revolution arrived? *Nature* 464: 674-675.
5. De Leon J et al. (2006). The AmpliChip CYP450 genotyping test: integrating a new clinical tool. *Mol Diagn Ther* 10: 135-151.
6. Encode Project Consortium. (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 447: 799-816.
7. Fackler JL, McGuire AL. (2009). Paving the way to personalized medicine: steps to successful implementation. Curr Pharmacogenomics *Person Med*. 7: 125
8. Hamburg MA, Collins FS. (2010). The path to personalized medicine. *NEJM* 363: 301-304.
9. Hoffmann W, Krafczyk-Korth J. (2011). Towards a unified concept of individualized medicine. *Pers Med* 8: 111-113.
10. http://www.genome.gov/sequencingcosts/ Accessed July 7, 2011.
11. Journal of Dental Education Supplement. (2008). Current and Clinical Training in Oral Health for Physicians and Dentists: Report of Panel 2 of the Macy Study. *Journal of Dental Education* Vol. 72, No. 2 supplement.
12. Kuska B. (1996). Wiskott-Aldrich syndrome is a "wonderful mystery." *J Natl Cancer Inst*. 88: 1258-1261.
13. Malamud D. (1992). Saliva as a diagnostic fluid. *Br Med J* 305: 207-208.
14. Miller CS et al. (2010). Current developments in salivary diagnostics. *Biomark Med* 4: 171-189.
15. Milne CP. (2010). Personalized medicine is playing a growing role in developmental pipelines. Tufts Center for the Study of Drug Development Impact Report 12: 1.
16. Moyer TP et al. (2009). Warfarin sensitivity genotyping: a review of the literature and summary of patient experience. *Mayo Clin Proc*. 84: 1079-1094.
17. Naylor S, Chen JY. (2010). Unraveling human complexity and disease with systems biology and personalized medicine. *Pers Med* 7: 275-28919.
18. Personalized Medicine Coalition. (2009). The Case for Personalized Medicine.
19. Steele FR. (2009). Personalized medicine: something old, something new. *Pers. Med* 6: 1-5.
20. Tokunaga E et al. (2006). Trastuzumab and breast cancer: developments and current status. *Int J Clin Onocol* 11: 199-208.
21. Venter C. (2010). Multiple Genomes Await. *Nature* 464; 676-677.
22. Vnencak-Jones CL. (1999). Molecular testing for inherited diseases. *Am J Clin Pathol* 12: S19-32.
23. Wang X et al. (2010). Genes and their effects on Dental Caries may differ between primary and permanent dentitions. *Caries Res* 44: 277-284;
24. Weigum et al. (2010). Nano-Bio-Chip sensor platform for examination of oral exfoliative cytology. *Cancer Prev Res*. 3: 518-528.
25. Wendell S et al. (2010). Taste genes associated with dental caries. *J Dent Res* 89: 1198-202.

Innate defense peptides in the oral cavity

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Key words: antimicrobial peptides, LL-37, innate immune response, bacteria, periodontal disease, Ghrelin, additive bactericidal effects

ABSTRACT

Antimicrobial peptides (AMPs), such as LL-37 and human beta-defensins, expressed in oral cavity are implicated to play pivotal innate immune protection functions. Especially, the importance of LL-37 in preventing the onset and progression of periodontal disease is best represented by morbus Kostmann and Papillon-Lefèvre syndromes. The pathogenesis of these diseases is marked by the failure of LL-37 to express, or the diminished production of bioactive LL-37, which permits the outgrowth of periodontal pathogens. However, it is widely accepted that the functionally active concentration of these peptides in both saliva and gingival crevice fluid (GCF) is too low to elicit bactericidal effects. Therefore, in order to harness commensal bacteria in the oral cavity and prevent infection by exogenous pathogens, there appears to be an unknown mechanism overriding the otherwise low concentration detected *in vivo* and allowing AMPs to perform their protective functions. This mini-review discusses the biologically cogent aspects of AMPs and also explores the novel mechanisms of AMPs in the context of 1) additive bactericidal effects between LL-37 and other AMPs and 2) Ghrelin, a novel class of AMP found in the oral cavity.

1) Host innate immune system produces antimicrobial peptides and proteins

Plants, insects and animals can produce Antimicrobial Peptides (AMP) and Antimicrobial Large Proteins (AMLP) to prevent infection from exogenous pathogens, as well as avoid opportunistic infections with commensal bacteria [1].Secretions of antimicrobial peptides and proteins are found in vertebrate skin, trachea, saliva and oral epithelia, where an abundance of microorganisms colonize. In contrast to AMLPs, such as lysozyme and lactoferrin (MW, 14 kDa and 75 kDa, respectively), AMPs are small proteins, generally between 12 and 50 amino acids in length (MW, 1.2 kDa - 5 kDa). The first peptide antibiotics were found in insects in 1981 [1]. In 1997, the production of peptide antibiotics (beta-defensins) by human skin epithelia was first discovered [2].Human alpha-defensin 1 (HNP-1) was the first AMP identified in saliva in 1998 [3].

2) Bactericidal AMPs, LL-37 and hBDs, are produced in oral cavity

Mucin, immunoglobulin, agglutinin, lysozyme and lactoferrin are the major AMLPs found in saliva [4]. Histatins, defensins and cathelicidin-type peptides are the major AMPs found in the oral cavity [5]. It has been demonstrated that these antimicrobial peptides and proteins protect the oral cavity from such periodontal pathogenic bacteria as *P. gingivalis* and *A. actinomycetemcomitans* [6-8]. The antimicrobial functions mediated by mucin, immunoglobulin and agglutinin are limited to the induction of bacterial aggregation, whereas lysozyme and lactoferrin can suppress the growth of bacteria [4]. In contrast to lysozyme and lactoferrin, most AMPs, including LL-37 and beta-defensins, possess true bactericidal effects. Human beta-defensins (hBDs) are prominently secreted by epithelial cells, including gingival epithelia. LL-37 (CAP18) is produced by both neutrophils and epithelial cells in the oral cavity. These antimicrobial peptides have a broad spectrum of bactericidal activity against Gram-positive and Gram-negative bacteria, as well as fungi, such as *Candida albicans*, and enveloped viruses, by permeabilizing anionic lipid bilayers of microorganisms, leading to the release of bacterial cytoplasmic contents [5]. We have also reported that the clinical isolates of MRSA are sensitive to both LL-37 and hBDs [9].

3) LL-37 plays a pivotal antimicrobial function in the oral innate immune system

The relevance of antimicrobial peptide LL-37 as a defense molecule in saliva was demonstrated by clinical cases of "morbus Kostmann," a syndrome which is characterized by severe congenital neutropenia accompanied by devastating periodontal disease [10]. While the neutropenia of patients with "morbus Kostmann" can be treated by the administration of recombinant GM-CSF, a cytokine that increases neutrophil production, patients still have a severe form of periodontitis as a consequence of the diminished production of LL-37 in saliva. A severe form of periodontitis is also found in Papillon-Lefèvre syndrome (PLS), a disease caused by a genetic disorder of cathepsin C [11]. Recent studies revealed that patients with PLS also lack the catalytic activities of the polymorphonuclear leukocyte (PMN)-derived serine proteinases

elastase, cathepsin G, and proteinase 3 [12]. Very interestingly, PMNs of PLS patients produce lower levels of LL-37 than healthy subjects, while still expressing a sufficient amount of hCAP-18, a proform of LL-37, possibly resulting from deficiency of serine proteinases that are capable of converting the PMN-derived hCAP-18 into LL-37. Since LL-37, but not hCAP-18, exerts antimicrobial activity, diminished production of antimicrobial LL-37 appears to be responsible for the outgrowth of *A. actinomycetemcomitans* in the gingival crevice of PLS patients [12].

In addition to the relevance of LL-37 in preventing the onset and progression of periodontal disease by its bactericidal effects, the high affinity of LL-37 for LPS plays a role in neutralizing the bioactivity of LPS [13]. Since the most virulent periodontal pathogens are Gram-negative bacteria which produce LPS, the neutralization of LPS by LL-37 is thought to play a relevant role in host protection from challenge by periodontal pathogens.

4) Multiple AMPs in concert elicit antimicrobial effects

The antibiotic concentration of these AMPs ranges from 1-100 ug/ml, as monitored by *in vitro* bactericidal assay. However, when a single species of AMP was assayed, the bactericidal effect mediated by LL-37 or hBDs was found to be attenuated in the physiological concentration of salt [7, 9]. Furthermore, the concentrations of AMPs detected in saliva and gingival crevice fluid ranges around 1-1000 ng/ml [14]. Under these conditions, only modest bactericidal effects of AMPs could be expected in the saliva and other microenvironments with a physiological concentration of salt. At the same time, however, our studies have indicated that the additive effects of multiple AMPs and neuropeptides, such as vasoactive intestinal peptide (VIP), can abrogate or mitigate the salt-sensitive bactericidal effect of LL-37 or hBDs [15]. Under the physiological condition of oral cavity, this means that the total antimicrobial activity in saliva and GCF could be higher than that estimated by *in vitro* assay. As such, the synergistic, or additive, bactericidal effects mediated by these multiple AMPs and neuropeptides in the oral cavity of patients with periodontal disease remain to be elucidated.

5) A novel class of AMP, Ghrelin (GHR), is produced in GCF and saliva

Ghrelin (GHR) was originally found as a cationic peptide hormone secreted from endocrine cells of the stomach and pancreas to stimulate hunger. Our group recently discovered that GHR secreted in saliva, as well as GCF, may play an anti-inflammatory role in the oral cavity [16]. Very interestingly, the amount of GHR detected in GCF was about 500-fold higher than that found in saliva [16]. Based on our experience in the study of anti-microbial peptides, unique chemical property of Ghrelin, i.e. abundance of positively charged amino acids (AA), arginine and lycine (25% of a total 28 AA), which is very similar to antimicrobial peptide, LL-37 (29% of a total 37 AA), lead us theorize that Ghrelin should possess anti-microbial activity because mechanism underlying antimicrobial activity by antimicrobial peptides is related to their strong positive charge which binds to the negatively charged cell wall of microorganisms. Indeed, our analysis using a bioinformatics tool implicated that Ghrelin may possess strong antimicrobial activity, according to the results that showed "net charge" of Ghrelin as strong as hBD2 or LL37, compared to other neuron-hormone peptides, such as Orexin B, VIP or Neuropeptide Y (Table 1). We additionally found that GHR may possess antimicrobial activity against *Escherichia coli*, as well as *S. aureus* (Figure 1). GHR also displayed additive effects on LL-37-mediated bactericidal activities against *E. coli* (Figure 1) and *S. aureus*. In contrast to LL-37, other AMPs, such as HNP-1, HBD-1 or HBD-4, did not show additive effects on GHR-mediated bactericidal effects (Figure 2. HBD-1), which suggests that secreted GHR in the saliva, as well as in GCF, can distinctively promote the bactericidal effects of LL-37. Furthermore, the additive effect of GHR on LL-37-mediated bactericidal activity was maintained in the presence of a physiological concentration of NaCl (150 mM). While the GHR's antimicrobial effects on periodontal

Table 1. Chemical properties of peptides

	#AA	Charge	IP
LL37*	37	6.0	11.1
hBD2*	41	5.8	9.2
Histatin 5*	24	5.6	10.7
Ghrelin	**28**	**5.1**	**11.5**
Orexin B**	28	3.1	12.1
VIP**	28	3.1	10.2
Neuropeptide Y**	36	0.1	7.6

*, antimicrobial peptide; **, neuropeptide;
#AA, number of amino acids; IP, Isoelectric point

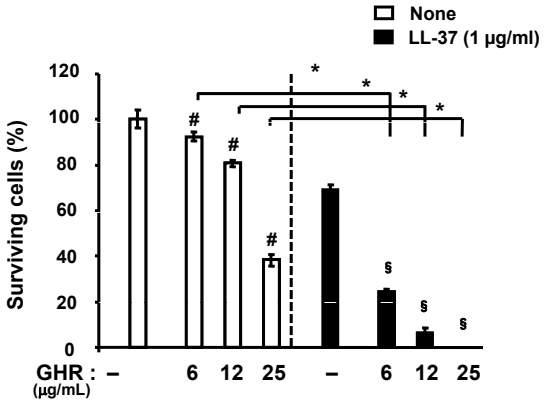

Figure 1: GHR demonstrated additive effects on the bactericidal activity mediated by LL-37 against *E. coli.*
Bacterial cell suspension in sodium phosphate buffer (NaPi, pH 7.4) with or without antimicrobial peptides (GHR and/or LL-37) was incubated for 2 hours at 37°C. The mixture was applied to BHI agar plate and incubated at 37°C overnight. Colony forming unit (CFU) was assessed by counting the number of bacterial colonies. The bactericidal effect was expressed as the ratio (%) of surviving cells incubated in the test solution compared to the one in the control NaPi. #, *, §, statistically significantly different by ANOVA; # non-treated control vs. GHR alone, *GHR alone vs. GHR+ LL-37 and § LL-37 alone vs. GHR + LL-37 ($P < 0.05$).

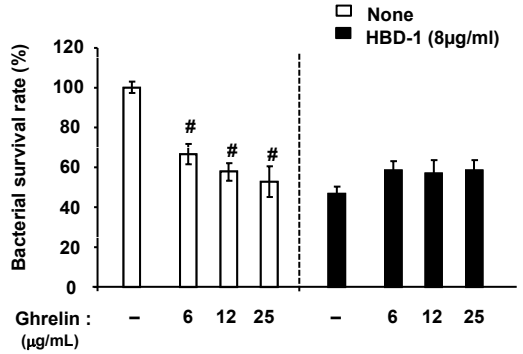

Figure 2. Lack of additive effects of HBD-1 on the bactericidal activity mediated by LL-37 against *E. coli*.
Following the methods noted in Figure 1, bactericidal activities against *E. coli* mediated by LL-37 with or without HBD-1 were determined. #, statistically significantly different by ANOVA; # non-treated control vs. GHR alone ($P < 0.05$).

pathogens and possible association between the amount of GHR in saliva/GCF and status of periodontal disease remain to be evaluated, these results indicated that GHR may function as an antimicrobial peptide in the physiological context of oral-mucosa, especially mediating strong antibacterial synergism with LL-37.

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REFERENCES

[1] Boman, H.G. (1995) Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 13, 61-92.

[2] Harder, J., Bartels, J., Christophers, E. and Schroder, J.M. (1997) A peptide antibiotic from human skin. Nature 387, 861.

[3] Mizukawa, N., Sugiyama, K., Fukunaga, J., Ueno, T., Mishima, K., Takagi, S. and Sugahara, T. (1998) Defensin-1, a peptide detected in the saliva of oral squamous cell carcinoma patients. Anticancer Res 18, 4645-4649.

[4] Van Nieuw Amerongen, A., Bolscher, J.G. and Veerman, E.C. (2004) Salivary proteins: protective and diagnostic value in cariology? Caries Res 38, 247-253.

[5] Bals, R. (2000) Epithelial antimicrobial peptides in host defense against infection. Respir Res 1, 141-150.

[6] Wakabayashi, H., Yamauchi, K., Kobayashi, T., Yaeshima, T., Iwatsuki, K. and Yoshie, H. (2009) Inhibitory effects of lactoferrin on growth and biofilm formation of Porphyromonas gingivalis and Prevotella intermedia. Antimicrob Agents Chemother 53, 3308-3316.

[7] Ouhara, K., Komatsuzawa, H., Yamada, S., Shiba, H., Fujiwara, T., Ohara, M., Sayama, K., Hashimoto, K., Kurihara, H. and Sugai, M. (2005) Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, {beta}-defensins and LL37, produced by human epithelial cells. J Antimicrob Chemother 55, 888-896.

[8] Murakami, Y., Nagata, H., Amano, A., Takagaki, M., Shizukuishi, S., Tsunemitsu, A. and Aimoto, S. (1991) Inhibitory effects of human salivary histatins and lysozyme on coaggregation between Porphyromonas gingivalis and Streptococcus mitis. Infect Immun 59, 3284-3286.

[9] Midorikawa, K., Ouhara, K., Komatsuzawa, H., Kawai, T., Yamada, S., Fujiwara, T., Yamazaki, K., Sayama, K., Taubman, M.A., Kurihara, H., Hashimoto, K. and Sugai, M. (2003) Staphylococcus aureus susceptibility to innate antimicrobial peptides, beta-defensins and CAP18, expressed by human keratinocytes. Infect Immun 71, 3730-3739.

[10] Putsep, K., Carlsson, G., Boman, H.G. and Andersson, M. (2002) Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. Lancet 360, 1144-1149.

[11] Toomes, C., James, J., Wood, A.J., Wu, C.L., McCormick, D., Lench, N., Hewitt, C., Moynihan, L., Roberts, E., Woods, C.G., Markham, A., Wong, M., Widmer, R., Ghaffar, K.A., Pemberton, M., Hussein, I.R., Temtamy, S.A., Davies, R., Read, A.P., Sloan, P., Dixon, M.J. and Thakker, N.S. (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. Nat Genet 23, 421-424.

[12] de Haar, S.F., Hiemstra, P.S., van Steenbergen, M.T., Everts, V. and Beertsen, W. (2006) Role of polymorphonuclear leukocyte-derived serine proteinases in defense against Actinobacillus actinomycetemcomitans. Infect Immun 74, 5284-5291.

[13] Nagaoka, I., Hirota, S., Niyonsaba, F., Hirata, M., Adachi, Y., Tamura, H., Tanaka, S. and Heumann, D. (2002) Augmentation of the lipopolysaccharide-neutralizing activities of human cathelicidin CAP18/LL-37-derived antimicrobial peptides by replacement with hydrophobic and cationic amino acid residues. Clin Diagn Lab Immunol 9, 972-982.

[14] Turkoglu, O., Emingil, G., Kutukculer, N. and Atilla, G. (2009) Gingival crevicular fluid levels of cathelicidin LL-37 and interleukin-18 in patients with chronic periodontitis. J Periodontol 80, 969-976.

[15] Ohta, K., Kajiya, M., Zhu, T., Nishi, H., Mawardi, H., Shin, J., Elbadawi, L., Kamata, N., Komatsuzawa, H. and Kawai, T. (2010) Additive effects of orexin B and vasoactive intestinal polypeptide on LL-37-mediated antimicrobial activities. J Neuroimmunol 233, 37-45.

[16] Ohta, K., Laborde, N.J., Kajiya, M., Shin, J., Zhu, T., Thondukolam, A.K., Min, C., Kamata, N., Karimbux, N.Y. and Kawai, T. (2011) Expression and possible immune-regulatory function of ghrelin in oral epithelium. J Dent Res Accepted for publication.

Education Session

Standardization and Mutual Recognition of Dental Education in Asia

Chulalongkorn University Faculty of Dentistry curriculum reform

Chulalongkorn University, Faculty of Dentistry, Dean

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Challenges of dental education in Asia: Advocating standardization and collaboration

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Chulalongkorn University Faculty of Dentistry curriculum reform

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Key words: Chulalongkorn, Dentistry, Curriculum, Reform, Competency, Education

ABSTRACT

Rationale

The public desires dentists who are more than just individuals who have passed their courses. They wish them to be safe, effective, and accountable clinicians. In other words, they want competent clinicians. Moreover, graduation is not the end point of their dental education. Due to changes in technology, more than half of the information dentists learn for treating patients is acquired after graduation. Thus, graduates need self directed learning, self assessment, and critical thinking skills. Therefore, the dental curriculum should be characterized in terms of its impact on students while focusing on outcomes of dental education i.e., the dental graduates themselves. These can be expressed as Competencies.

Objective

To reform the undergraduate dental curriculum from a discipline based curriculum to one which is competency based.

Design

First, the perception of the need for curriculum changes was surveyed and, on this basis, the decision was made to enact changes. Competency components and contents were established and analyzed. New curriculum structure, subjects, and course descriptions were drafted. This was followed by composing an entire revised program with detailed descriptions from year one through year six of dental education.

Results

We enacted major improvements in all aspects of our curriculum.

With clear goals, objectives, and relevant content suitable for optimal teaching and learning experiences, we created a well organized curriculum with systematic and comprehensive competency evaluations.

Conclusions

We achieved the highest quality in dental education by developing an educational program suitable for producing general dental practitioners in the 21st century. These are competent practitioners who are caring, ethical, and ready for future self-directed learning.

In the past 20 years, many changes have occurred influencing the evolution of dental education, including changes in patient expectations, technology, and educational techniques. At the Faculty of Dentistry, Chulalongkorn University, we recognized the need to modify and update our curriculum in order to continue to generate the highest quality dental clinicians possible. The basis for our curriculum reform was the development and evaluation of student competency. Competency can be defined as the characteristics and capabilities required of dental school graduates. Competency blends scientific knowledge, clinical skills, and values into an integrated response to circumstances encountered in professional general dental practice. This requires a combination of efficiency, accuracy, and consistency. However this does not mean performance at the highest level possible. Thus, dental competency is an amalgamation of the abilities essential to begin the unsupervised practice of dentistry, consisting of behaviors and skills expected of the nascent independent practitioner. What comprises competency is based on developing statements describing the dental graduates which are grounded in function. Precise definitions of trainee outcomes focusing on what students need to learn in order to perform in independent clinical practice, with hierarchically sequenced and interdisciplinary educational modules, including competency assessment are the characteristics of a competency based dental curricula.

Our concern with the previous curriculum was that it was heavily discipline-centric with each department working independently of each other. This emphasized individual disciplines rather than the holistic practice of general dentistry. There was limited integration between disciplines and some of the same information was being taught in multiple courses, which was not a good use of the limited time available. Certain specialized content received undue attention for the pre-doctoral level, as well. The style of teaching was teacher-centered or didactic style with little or no student participation. There were insufficient teaching-learning activities to enable students to gain a deeper level of comprehension. The style of learning was rote memorization rather than understanding, and knowledge was compartmentalized as students studied subjects separately. Consequently,

students had insufficient opportunity to consolidate concepts and developed technical skills without sufficient development of clinical reasoning. The curriculum was deficient in comprehensive dentistry e.g. total patient care, communication, management, and team work. Moreover, evaluation techniques were largely limited to separate discipline-based exams testing factual knowledge instead of comprehension or the capacity to solve problems. Additionally, students' practical skills were evaluated based on ideal form rather than what is clinically acceptable.

Other factors driving the need for curriculum revision were external pressure from national education standards and quality assessment evaluations, as well as health care reform and dental school accreditation requirements to update our curriculum. The expansion in patients' oral health needs from symptomatic and palliative treatment to elective esthetic based treatment, combined with rapid changes in socioeconomics and considerable advances in the sciences, clinical techniques and materials, and information technology motivated us to improve the curriculum as well.

In 2002 our dental school's Executive and Curriculum Committees moved to restructure the existing undergraduate curriculum into a competency-based curriculum. The new curriculum was launched in 2007. The first class of students training in the revised program started their 5th year in the new curriculum in June 2011.

The curriculum revision process began with an assessment of needs, and the decision to change the curriculum. We then moved into setting competency standards, drafting competency components and content analysis, followed by generating a new curriculum structure with course subjects and descriptions. The process was completed with the production of a final written version of the curriculum with detailed descriptions for each year. The procedure for improvement included developing a set of statements defining graduates of various educational experiences i.e. undergraduate versus post graduate, reviewing and refining course content and learning activities, setting course sequences to coincide with competency examinations and restructuring the methods for evaluating students, especially in regards to competency.

Curriculum content was refined to include the use of content directly relevant to the desired competencies. Knowledge was incorporated from separate subjects resulting in greater integration between subjects. Subject matter was additionally revised to include updated materials and a better sequencing of topics. We reduced repetition of information while acknowledging its significance to each discipline and eliminated outdated and nonessential materials, while still recognizing the value of the classic literature. There was an increased emphasis in the areas of professionalism, communication, interpersonal skills, practice management, and comprehensive patient care. Opportunities were also provided for interested students to study additional topics or techniques.

Teaching and learning strategies were also reformed including adding more active learning into the curriculum thereby instilling into students the practice of self-directed learning. The sequence of learning activities were more closely integrated, with didactic theory, preclinical laboratories, and clinical experiences being enhanced by earlier clinical exposure. We also improved the evaluation processes both of students and the program.

Competency was based on four components. First were performance criteria reflecting the standard of performance required for the successful achievement of the elements of competence. The second component consisted of range indicators describing levels of competencies such as the different abilities required of undergraduate and post graduate students. The third component was knowledge requirements specifying the essential knowledge, attitudes, and practice skills which students need to learn and be trained in, to be considered competent. The fourth component was performance evidence describing the clinical skills the student must demonstrate to demonstrate competency.

The content of our curriculum was organized into 6 areas of concentration. These are professional development and comprehensive patient care; dental public health; biomedical science related to dentistry; promotion, prevention, treatment, and rehabilitation of the dentition; promotion, prevention, treatment, and rehabilitation of oral soft tissue and the periodontium; and promotion, prevention, treatment, and rehabilitation of the craniofacial complex.

Dental School Faculty seminars were used as a platform for presenting data to consider the proportion of time allocated to each group of subjects. Seminar topics also included the results of the analysis of needs, proposing hours of education for various disciplines, and data from other schools around the world for time allocated to each subject. Other seminars discussed the proportion of educational hours between groups of subjects, and analyzed which topics should be taught each year. Faculty member's skills for implementing the new curriculum were developed through workshops on topics such as problem-based learning, techniques for evaluation of students, how to analyze components of competencies, and active learning.

In conclusion, our new curriculum features 232 credit hours with decreased basic science subjects and closer integration of theory, Preclinical Lab, and Clinic. The educational content was arranged by areas of concentration and taught utilizing both passive and active learning techniques. There is at least one session per week reserved for self-directed learning. A major improvement is the incorporation of earlier clinical exposure incorporating orderly learning and training experiences progressing from simple to more difficult tasks. Additionally, students work with, and are mentored by, more senior clinical students. The new curriculum also provides added experience with advanced cases and higher level research projects.

Challenges of dental education in Asia: Advocating standardization and collaboration

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Key words: -standardisation, recognition; dental education, Asia

ABSTRACT

Globalisation is the intensification of global flows of capital, goods, ideas and people across borders. ASEAN members of the ASEAN Free Trade Area (AFTA) have signed a mutual agreement to realize a liberalized and integrated ASEAN economic community by 2015. For the purpose of enhancing this mobility, there is need for mutual recognition of national professional qualifications with the standardization of training and skills of professionals with pooled resources and information available to all countries. The South East Asian Association for Dental Education (SEAADE) can play a key role in quality assurance and accreditation process thus facilitating mobility of dental professionals in Asia.

INTRODUCTION

Globalisation is a broad term that refers to the intensification of connectivity, integration and interdependence of economies, societies, technologies, cultures, political and ecological spheres across the world with outcomes measured in terms of economic growth, increased personal incomes, improved living conditions and liberal democracy for the betterment of humanity. Globalization of healthcare (UNDP, 1997) requires healthcare professionals to be more competitive and practicing evidence-based healthcare, be more vigilant, transparent and accountable in addition to striving to ensure and maintain recognized standard of care. This requires strong national health policies, institutions, regulations and programs.

In the Asian region, the economic ministers of the Association of Southeast Asian Nations (ASEAN) in 1995, agreed to the establishment of an ASEAN Common Market (AEC) with the aim of allowing continued growth and prosperity in the region, enabling it to withstand global competition and this was targeted to be established by 2020 (AFTA, 2009). The implications of globalization of healthcare is twofold ie. the migration of health professionals and the standardization/mutual recognition of dental practices.

A common ASEAN dental market signifies a free exchange of goods and services in the health care sector where healthcare groups can be set up in any ASEAN country regulated by local laws and regulation. In line with this, the national licensing rules should be uniform for locals as for foreigners from the ASEAN countries (Sarjeet, 2008). Whilst it is obvious that these globalization moves have an impact on the dental profession (Berry, 2006), its implication on the dental education sector remains to be fully realized. Working together as healthcare players and providers require mutual recognition of standards of oral healthcare. In the globalization of dental education, dental educators can work together to identify common challenges, share experiences and pool intellectual resources.

Against this background the key aim of this paper is to address some of the issues of standardizing and mutual recognition of the dental education in the Asian countries that would address the incongruence of the dental curriculum, standardization of assessment of competencies, collaboration and pooling of resources to be made available to all.

Incongruence of the Dental Curriculum

In dentistry there are definable clinical skills and competences without which a dentist would be unable to provide basic dental care and without which patient safety could not be assured. In Malaysia, there is currently no officially established set of core competencies for the dental curriculum. In addition, many of the dental schools in Malaysia are still using the assessment formats which do not capture the necessary skills required of a new dental graduate.

Across the country as well as in the Asian region, there are ongoing efforts to develop a mutual set of core curriculum where content and competencies that are fundamental to the accomplishment of the professional profile and learning outcomes can be defined. Compounding this incongruence is the multiplicity of learning context and educational methodologies which sometimes lack the evidence-based foundation. A convergence will allow for mutual recognition of undergraduate studies from dental schools across the region. More importantly it will ensure that patients receive uniform standards of oral healthcare.

ASSESSMENT

Assessment represents a critical component of education in measuring the successful achievement of the required skills, knowledge and affective domains including professional values that define the competent practice of dentistry. A clear and objective assessment process will serve as a springboard for students to adopt a posi-

tive approach to effective independent practices, reflective learning and life-long learning after graduation (ADEE, 2010). The scope of assessment is vital in order to capture the essential requirements for a competent dental practitioner. Effective assessment includes both formative and summative assessment practices with different aims, aligned to program design, linking content with methods of teaching and learning, expected outcomes and objectives. There is also a need to employ a wide variety of assessment methods that would achieve a variety of end points indicative of an appropriate level of competency, utilizing criteria based on a standard set of achievement and which reflect the intended learning outcome. As a general rule the higher the level of performance (Bloom, 1956) that can be assessed, the more valid and useful the assessment will be.

Competencies for Dental Education

Competency commonly refers to the qualifications expected of an individual who is ready to begin independent practice of a profession such as dentistry and includes the skills, knowledge and values necessary for the successful conduct of that activity (ADEA, 1997; CDA, 1998). It is an outcome measure of the quality of the educational process and is reflective of the professional development in its entirety. The concept of levels of competencies is important when designing courses for the development of both core and specialist skills. The former is essential for the practice of dentistry at the primary care level. The basic level of competencies proposed must be supported by knowledge of basic biomedical, behavioral and dental sciences; psychomotor and cognitive skills; and also ethical, social and professional values; the integration of which marks the key for the successful implementation of competencies. Models of competencies have been developed for use in Europe and North America (ADEE, 2010; ADEA, 1997; CDA 1998).

The merits of preparing a dental curriculum with the stated levels of competencies are twofold ie. it defines the core content and competencies of the dental graduates as well as serving as the basis for outcomes assessment. The student must be appropriately assessed to demonstrate that he has achieved the stated competencies to qualify for graduation and entry into the profession. The development of a set of core competencies will promote the adoption of common curriculum components and similar learning experiences for students leading to a more standardized approach to assessment of students' competencies and hence a more consistent quality of oral healthcare services across the region. These competencies should be reviewed periodically to meet the changing demands of patients and future practice environments.

Southeast Asian Association of Dental Education (SEAADE)

The SEAADE aims to promote the advancement of dental education and research in all institutions, facilitate cooperative effort and achievement by collaboration among dental educators in this region, to stimulate the production, exchange and dissemination of ideas and information among a wide spectrum of educators, to advise and provide consultations on dental education programs (SEAADE, 2011). As the primary regional association focused on dental education SEAADE has established a Peer Review and Consultation service to assist dental institutions in the region leading to progressive strengthening of the dental institutions in the region to achieve and maintain high quality standards and enhancement of collaboration amongst dental educators in the region.

Being a neutral body and having in place a tool, SEAADE can play an enabling role between the nations. This could be extended to include accreditation of dental programs of institutions for the region by having representatives from the professional bodies to meet and agree on expectations that will act as guidelines. Therefore SEAADE requires AFTA central body to empower SEAADE to implement these initiatives through national and regional networking for quality assurance in the mobility of dental professionals in Asia.

REFERENCE

American Dental Education Association (ADEA) (1997). Competencies for the new dentist, J Dent Educ 61 (7): 556-8

ASEAN Free Trade Area (AFTA) - An Update. At: http://www.aseansec.org/12021.htm. Accessed Aug 10, 2011.

Association for Dental Education in Europe (ADEE): Curriculum structure, Content, Learning and Assessment in European Undergraduate Dental Education – Update 2010. At: www.adee.org. Accessed: Aug 10, 2011.

Berry J. Globalization and its implication for dentistry. ADA News (2006). At www.ada.org/prof/resources/pubs/adanews/adanewasarticle.asp?articleid=1883. Accessed: Aug 10, 2011.

Bloom BS. Taxonomy of educational objectives, Handbook I (1956): Cognitive domain, New York, Longman, Green and Co.

Canadian Dental Association (CDA) (1998). Report of the Task Force on Dental Education prepared by a Steering Committee of the Council on Education.

Sarjeet SS. Implications of AFTA for medical associations and the medical profession. MMA News (2008), Vol.38 (11): pgs 13-14.

United Nations Development Program (UNDP) (1997). "Globalization—Poor Nations, Poor People." pp. 82-93 in Human Development Report 1997. New York: Oxford University Press.

Science Session

Transplantable Cells and Biomaterials for Regenerative Medicine

Cell surface modification with polymers for biomedical studies

Kyoto University
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Tooth regenerative therapy as a future dental treatment

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Gingiva-derived iPS cells: A promising tool for dental applications

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Key words: cell, surface modification, islets of Langerhans

ABSTRACT

We overview new methodologies for using amphiphilic polymers to modify the surfaces of cells and tissues. Coating the cell surface with amphiphilic polymers that can capture and immobilize bioactive substances or cells represents a promising approach for clinical applications, particularly cellular therapies.

1. Cell surface modifications

Cell surfaces can be modified in a number of ways, including covalent conjugation of polymers to amino groups on membrane proteins [1-4], electrostatic interaction between cationic polymers and a negatively charged cell surface [5-8], and hydrophobic interactions that anchor long alkyl chains of amphiphilic polymers to the lipid bilayer of the cell membrane [9-25]. These methods have been used to immobilize various functional groups and bioactive substances onto the cell surface. The covalent conjugation method is expected to impair membrane protein functions because polymers are attached by crosslinking to the amino groups on membrane proteins. Therefore, the extent of this reaction should be carefully controlled. The electrostatic interaction is performed by simply adding a cationic polymer solution to a cell suspension. However, most cationic polymers are cytotoxic; therefore, this treatment causes deterioration or death to most cell types. In contrast, the hydrophobic interaction can be performed by simply adding amphiphilic polymers with long alkyl chains to a cell suspension. This has not caused any major damage to cell function or integrity. Our group has extensively studied cell surface modifications with amphiphilic polymers [9-25].

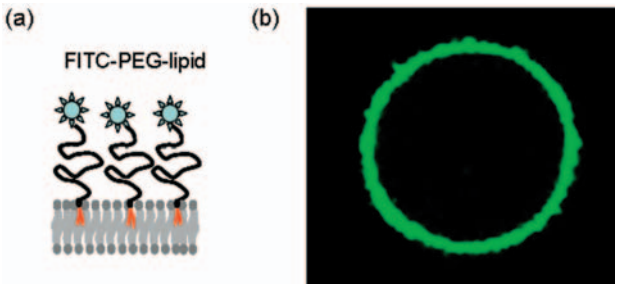

Fig. 1. Surface modification of cells with PEG-lipid.
(a) Schematic representation of surface modification of a CCRF-CEM cell with FITC-PEG-lipid, (b) Confocal laser scanning microscopic image of an modified CCRF-CEM cell.

2. Cell surface modifications with amphiphilic polymers

Amphiphilic polymers are typically derived by conjugating polyethylene glycol (PEG) to a phospholipid (PEG-lipid) [16, 17]. When a PEG-lipid solution is added to a cell suspension, the hydrophobic alkyl chains of the PEG-lipid spontaneously form hydrophobic interactions with the lipid bilayer of the cell membrane (Fig. 1a). This spontaneous anchoring was demonstrated with a human cell line derived from T cell leukemia cells (CCRF-CEM). A solution of fluorescein isothiocyanate (FITC)-conjugated PEG-lipid was added to a suspension of CCRF-CEM. Under a confocal laser scanning microscope, the bright fluorescence from FITC was observed at the periphery of all cells (Fig. 1b). This indicated that PEG-lipids had lodged on the cell surface. The retention time of PEG-lipids on cell membranes can be controlled by adjusting the length of the lipid alkyl chain. The dissociation rate of PEG-lipid was much slower with long than with short hydrophobic domains [10].

Proteins can be immobilized on the cell surface with the use of a short, single stranded DNA (ssDNA) attached to the end of a PEG chain (ssDNA-PEG-lipid) (Fig. 2) [13-15, 18, 24]. First, an ssDNA-PEG-lipid is prepared by conjugating maleimide-PEG-lipid with an ssDNA that carries a thiol group. While, a protein is modified with a hetero-bifunctional cross-linker, sulfo-EMCS (*N*-(6-maleimidocaproyloxy)sulfosuccinimide); next, it is treated with a ssDNA' that is complementary to the ssDNA on the PEG-lipid. Figure 2 shows a schematic of the procedure, where the ssDNA and ssDNA' are oligo (deoxythymidine) (oligo(dT)$_{20}$) and oligo(deoxyadenine) (oligo(dA)$_{20}$), respectively. The cells with oligo(dT)$_{20}$ attached are exposed to the protein with the oligo(dA)$_{20}$ attached. The protein is immobilized on the cell through hybridization between oligo(dT)$_{20}$ and oligo(dA)$_{20}$.

3. Immobilization of bioactive substances on an islet surface

Cell transplantation has shown promise as a method for treating serious diseases. Various kinds of pluripotent stem cells have been developed or identified, including embryonic stem (ES) cells, induced pluripotent stem

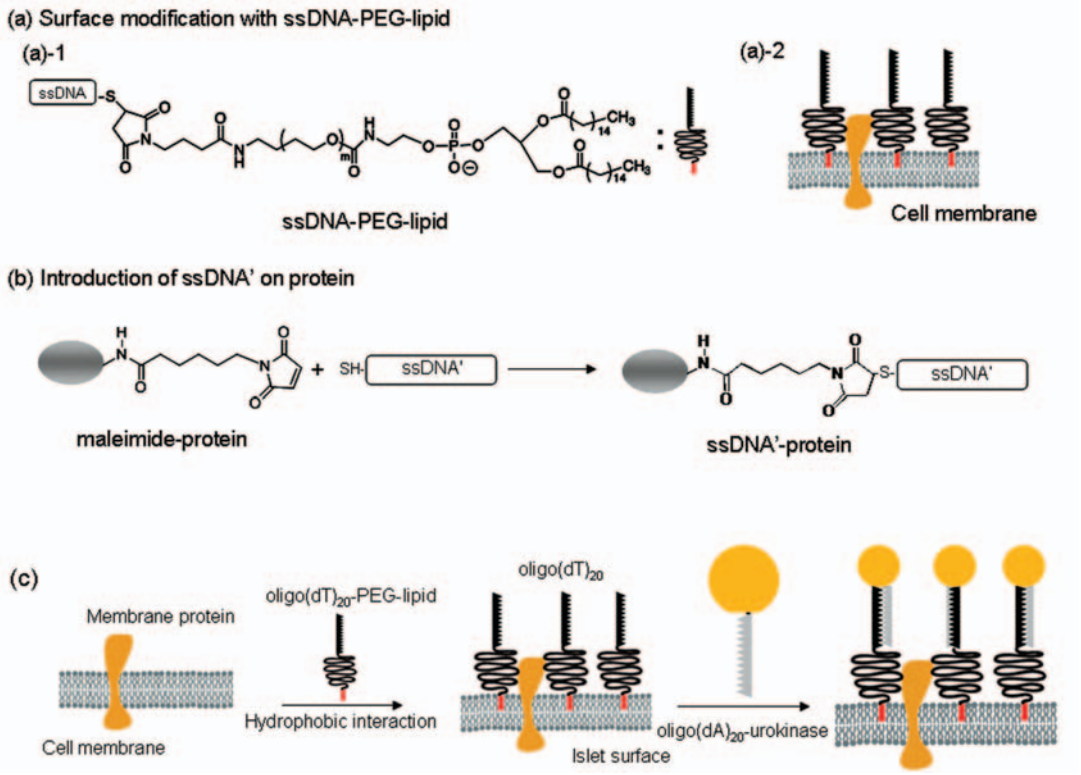

Fig. 2. Immobilization of urokinase protein on the surfaces of islet cells.
(a-1) Chemical structure of DNA-conjugated PEG-phospholipid (DNA-PEG-lipid); (a-2) ssDNA-PEG-lipid anchoring to the cell membrane. (b) Introduction of a complementary ssDNA onto urokinase protein, which was first modified with a madeimide group by EMCS. (c) Schematic representation of urokinase-immobilization through DNA hybridization.

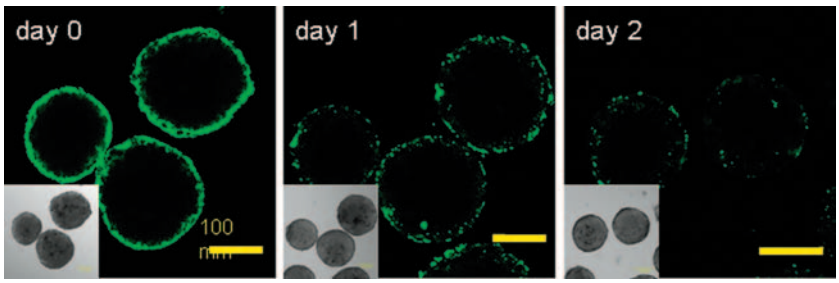

Fig. 3. Confocal laser scanning microscope images of islets with urokinase (UK) immobilized on the membrane.
The green signal indicates positive immunostaining for UK. (a) Islets were modified with oligo(dT)$_{20}$-PEG-lipid (C16) then, oligo(dA)$_{20}$-UK was added to the media. Scale bar: 100 μm.

(iPS) cells, and mesenchymal stem cells. Moreover, the differentiation of stem cells to functional cells has been extensively studied.

Previous studies have demonstrated that the transplantation of insulin secreting cells, that is islet of Langerhans cells (islets), could successfully treat type 1 diabetes. Islets are insulin secreting cells found in the pancreas. Over two hundred patients with type 1 diabetes have been clinically treated with islet transplantation. To cure the disease, a single patient typically requires islets from several donors, due to the destruction of islets just after transplantation. In the clinical setting, islets are infused into the liver through the portal vein. Exposure of islets to the blood activates blood coagulation and complement systems, which induce non-specific inflammatory reactions or instant blood-mediated inflammatory reactions (IBMIR). These host defense mechanisms destroy donor islets because they are considered foreign bodies. Anticoagulants, including aspirin, heparin, and dextran sulfate, are typically administered to inhibit blood coagulation. However, systemic infusion of these drugs increases bleeding. The optimal approach would be to prevent blood coagulation at the islet. Recent studies have been able to immobilize various bioactive substances, like heparin, urokinase, thrombomodulin, and the soluble domain of human complement receptor 1 (sCR1), on islets in attempts to control local activation of the blood coagulation and complement

systems [9, 11, 14, 16-18, 20, 24].

As an example, we will describe immobilization of the fibrinolytic enzyme, urokinase (UK), on the islet surface (Fig. 3) [14, 18]. UK could be immobilized on islets through ssDNA hybridization of oligo(dT)$_{20}$-PEG-lipid and oligo(dA)$_{20}$-UK. When the oligo(dT)$_{20}$-PEG-lipid was added to a suspension of islets, the lipid moiety spontaneously anchored to the lipid bilayer of the cell membrane through hydrophobic interactions. The oligo(dT)$_{20}$ segment was exposed on the cell surface, which made it accessible for conjugation with the oligo(dA)$_{20}$ on UK.

UK is a serine protease that activates plasminogen to plasmin. Plasmin dissolves the fibrin in blood clots. The attachment of UK to the islet surface was expected to dissolve blood clots that surrounded the islets in the liver; thus, IBMIR could be inhibited in the initial stages. A fibrin plate–based assay was performed to assess the function of the UK attached to the islets. Fifty islets with/without immobilization of UK were spotted onto a fibrin gel plate. After incubation, transparent areas around the spots indicated UK dissolution of the fibrin. Figure 4 shows the fibrin plate at 14 h after spotting the islets. Larger transparent areas were observed around the UK-islets compared to those around the unmodified islets (Fig. 4). These indicated that the immobilized UK retained its activity on the islets. UK-islets were also tested after 2 days of culture in the presence of serum (Fig. 4). UK activity rapidly decreased with 2 days in culture. The morphology of all islets after modification with UK was well maintained after 7 days of culture. Islets with UK maintained the ability to regulate insulin release in response to changes in glucose concentration (data not shown). We also performed transplantation of UK-islets by transfusion to the liver through the portal vein [18]. The transplantation results indicated that donor islets were rescued from host defenses by attaching UK to their surfaces. It remains to be determined how long UK-islets can maintain the inhibition of IBMIR; however, these data suggested that UK immobilization on islets is a promising approach for islet transplantation.

4.3 Encapsulation of islets with living cells

The histocompatibility and blood compatibility of donor islets can be significantly improved by enclosing them inside a capsule made of the patient's vascular endothelial cells. The ssDNA-PEG-lipid method was utilized to enclose islets with living cells [24]. The method is schematically shown in Fig. 5. Oligo(dT)$_{20}$ was introduced onto the surface of HEK293 cells with an oligo(dT)$_{20}$-PEG-lipid, and oligo(dA)$_{20}$ was introduced onto the surface of islets with an oligo(dA)$_{20}$-PEG-lipid. Then, the oligo(dA)$_{20}$-islets were mixed with the oligo(dT)$_{20}$-HEK293 cells. The HEK293 cells were immobilized on the islet surface through DNA hybridization, as shown in Fig. 5b. Although the HEK293 cells existed as single cells on the islet just after immobilization, the surface of islets were completely covered with a cell layer after 3 days in culture (Fig. 5). No central necrosis of the islet cells was observed. Immunostaining showed that insulin remained inside the islets after culturing for 3 days. Furthermore, after cell encapsulation, insulin secretion in response to glucose stimulation was well maintained (data not shown). This technique will greatly facilitate islet transplantation for treating type 1 diabetes.

REFERENCES

1. Contreras Jl, Xie D, Mays J et al. (2004) A novel approach to xenotransplantation combining surface engineering and genetic modification of isolated adult porcine islets. Surgery 136: 537-547
2. Hashemi-Najafabadi S, Vasheghani-Farahani E, Shojaosadati SA et al. (2006) A method to optimize

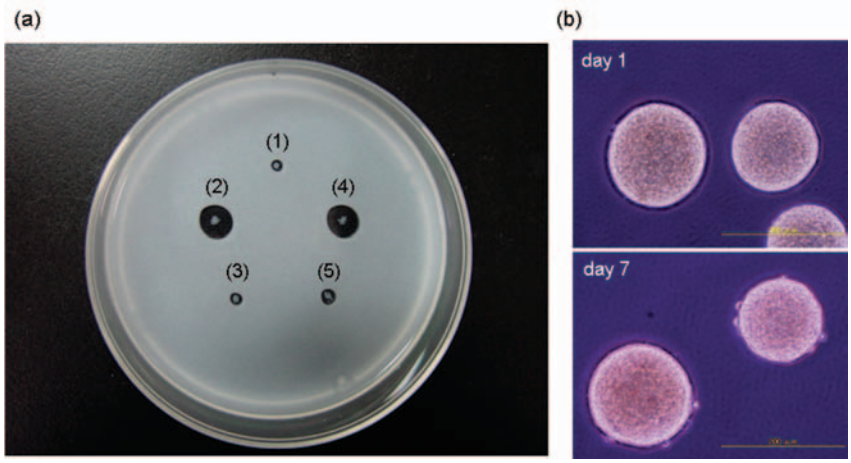

Fig. 4. Islets with immobilized urokinase (UK-islets) were tested for the ability to dissolve fibrin.
(a) Fibrin in the plate gel medium was dissolved by UK-islets (clear areas). Fifty islets were applied to each spot, and the plate was observed after incubation at 37 °C for 14 h. (1) untreated islets; (2) UK-islets (with oligo(dT)$_{20}$-PEG-lipid (C16)), just after preparation; (3) UK-islets (with oligo(dT)$_{20}$-PEG-lipid (C16)) lost activity after 2 days in culture; (4) UK-islets (with oligo(dT)$_{20}$-PEG-lipid (C18)), just after preparation; and (5) UK-islets (with oligo(dT)$_{20}$-PEG-lipid (C16)) lost activity after 2 days in culture. (b) Morphology of UK-islets after 1day and 7 days of culture.

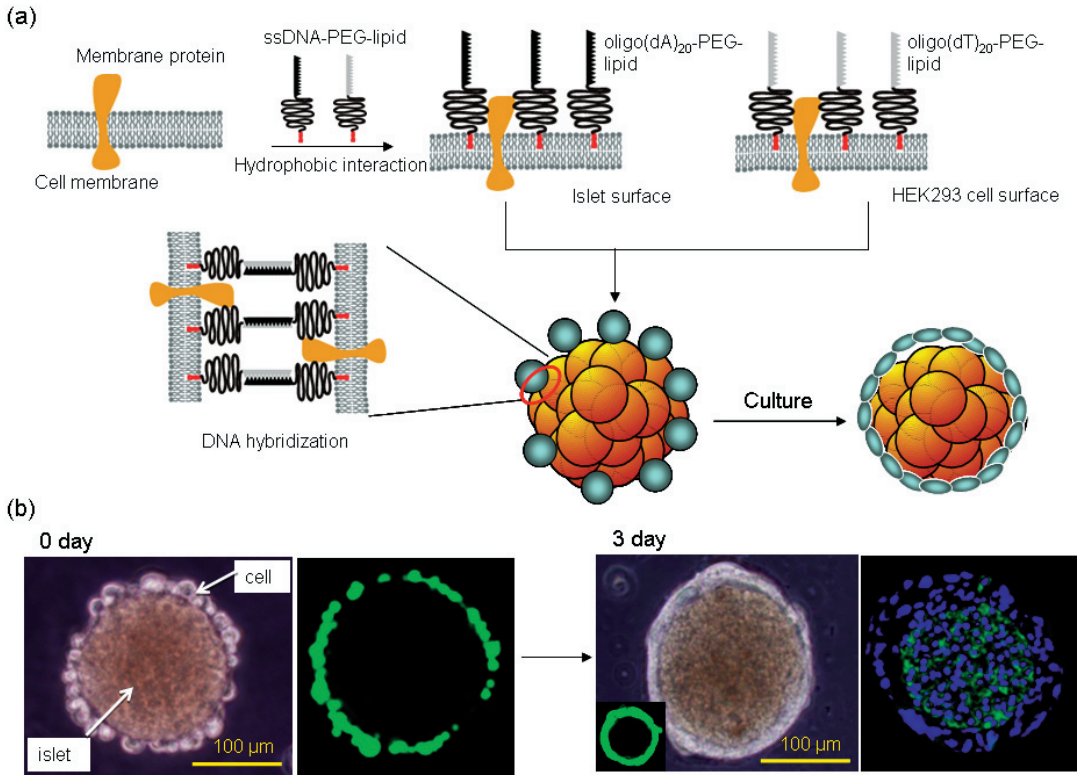

Fig. 5. Islet encapsulation within living HEK293 cells that express green fluorescent protein (GFP). (a) Schematic illustration. Islets are enclosed within a capsule of HEK293 cells (that express GFP) by introducing surface modifications of complementary single-stranded DNAs. Islets modified with oligo(dT)20-PEG-lipid are combined with HEK293 cells that have oligo(dA)20-PEG-lipid immobilized on the surface. DNA hybridization immobilizes the HEK293 cells to the surface of the islets. After 3 days in culture, islets are completely encapsulated within HEK293 cells. (b) Phase contrast (left panels) and fluorescence images (right panels) of islets with attached HEK293 cells. (0 days) GFP-HEK cells immobilized to islets observed with a confocal laser-scanning microscope; (3 days) Frozen sections of islets with attached GFP-HEK cells were stained with Alexa 488-labeled anti-insulin antibody (green) and Hoechst 33342 dye (blue) for nuclear staining.

PEG-coating of red blood cells. Bioconjug Chem 17: 1288-1293
3. Nacharaju P, Boctor FN, Manjula BN et al. (2005) Surface decoration of red blood cells with maleimidophenyl-polyethylene glycol facilitated by thiolation with iminothiolane: an approach to mask A, B, and D antigens to generate universal red blood cells. Transfusion 45: 374-383
4. Scott MD, Murad KL, Koumpouras F et al. (1997) Chemical camouflage of antigenic determinants: stealth erythrocytes. Proc Natl Acad Sci USA 94: 7566-7571
5. Elbert DL, Herbert CB, Hubbell JA (1999) Thin polymer layers formed by polyelectrolyte multilayer techniques on biological surfaces. Langmuir 15: 5355-5362
6. Germain M, Balaguer P, Nicolas Jc et al. (2006) Protection of mammalian cell used in biosensors by coating with a polyelectrolyte shell. Biosensors & Bioelectronics 21: 1566-1573
7. Krol S, Del Guerra S, Grupillo M et al. (2006) Multilayer nanoencapsulation. New approach for immune protection of human pancreatic islets. Nano Lett 6: 1933-1939
8. Veerabadran Ng, Goli Pl, Stewart-Clark Ss et al. (2007) Nanoencapsulation of stem cells within polyelectrolyte multilayer shells. Macromol Biosci 7: 877-882
9. Chen H, Teramura Y, Iwata H (2011) Co-immobilization of urokinase and thrombomodulin on islet surfaces by poly(ethylene glycol)-conjugated phospholipid. J Control Release 150: 229-234
10. Inui O, Teramura Y, Iwata H (2010) Retention dynamics of amphiphilic polymers PEG-lipids and PVA-Alkyl on the cell surface. ACS Appl Mater Interfaces 2: 1514-1520
11. Luan NM, Teramura Y, Iwata H (2011) Immobilization of soluble complement receptor 1 on islets. Biomaterials 32: 4539-4545
12. Miura S, Teramura Y, Iwata H (2006) Encapsulation of islets with ultra-thin polyion complex membrane through poly(ethylene glycol)-phospholipids anchored to cell membrane. Biomaterials 27: 5828-5835
13. Sakurai K, Teramura Y, Iwata H (2011) Cells immobilized on patterns printed in DNA by an inkjet printer. Biomaterials 32: 3596-3602
14. Takemoto N, Teramura Y, Iwata H (2011) Islet surface modification with urokinase through DNA hybridization. Bioconjug Chem 22: 673-678
15. Teramura Y, Chen H, Kawamoto T et al. (2010)

Control of cell attachment through polyDNA hybridization. Biomaterials 31: 2229-2235
16. Teramura Y, Iwata H (2010) Bioartificial pancreas microencapsulation and conformal coating of islet of Langerhans. Adv Drug Deliv Rev 62: 827-840
17. Teramura Y, Iwata H (2010) Cell surface modification with polymers for biomedical studies. Soft Matter 6: 1081-1091
18. Teramura Y, Iwata H (2011) Improvement of graft survival by surface modification with poly(ethylene glycol)-lipid and urokinase in intraportal islet transplantation. Transplantation 91: 271-278
19. Teramura Y, Iwata H (2009) Islet encapsulation with living cells for improvement of biocompatibility. Biomaterials 30: 2270-2275
20. Teramura Y, Iwata H (2008) Islets surface modification prevents blood-mediated inflammatory responses. Bioconjug Chem 19: 1389-1395
21. Teramura Y, Iwata H (2009) Surface modification of islets with PEG-lipid for improvement of graft survival in intraportal transplantation. Transplantation 88: 624-630
22. Teramura Y, Kaneda Y, Iwata H (2007) Islet-encapsulation in ultra-thin layer-by-layer membranes of poly(vinyl alcohol) anchored to poly(ethylene glycol)-lipids in the cell membrane. Biomaterials 28: 4818-4825
23. Teramura Y, Kaneda Y, Totani T et al. (2008) Behavior of synthetic polymers immobilized on a cell membrane. Biomaterials 29: 1345-1355
24. Teramura Y, Minh Ln, Kawamoto T et al. (2010) Microencapsulation of islets with living cells using polyDNA-PEG-lipid conjugate. Bioconjug Chem 21: 792-796
25. Totani T, Teramura Y, Iwata H (2008) Immobilization of urokinase on the islet surface by amphiphilic poly(vinyl alcohol) that carries alkyl side chains. Biomaterials 29: 2878-2883

Tooth regenerative therapy as a future dental treatment

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Key words: tooth regenerative therapy, tooth germ, bioengineered tooth, tooth replacement, transplantation

ABSTRACT

Damage, loss or the onset of disease in teeth, including dental caries and periodontal disease, can cause fundamental problems for oral functions and associated health issues. The development of tooth regenerative therapy for tissue repair, such as dental pulp or periodontal tissues, and whole tooth replacement is in progress as a novel dental treatment approach with the potential to fully recover tooth functions. Whole tooth replacement therapy for the loss of tooth as a form of bioengineered organ replacement has been challenged from the transplantations of bioengineered tooth germ and/or unit including bioengineered tooth, periodontal ligament and alveolar bone, in tooth loss site of oral environment. This paper herein reviews the recent findings and technologies underpinning tooth regenerative therapy.

INTRODUCTION

Oral functions such as enunciation, mastication and occlusion, are an important aspect of good health and quality of life (Proffit, 2007). The tooth is an ectodermal organ induced by typical and reciprocal epithelial and mesenchymal interactions and has unique structures such as dentin, enamel, cementium, pulp tissues and periodontal tissue comprising periodontal ligament and alveolar bone (Ikeda & Tsuji, 2008; Pispa &Thesleff, 2003). Damage, loss and the onset of disease in teeth, including dental caries and periodontal disease, cause fundamental problems for oral functions such as enunciation, mastication and occlusion, and associated health issues (Proffit, 2007). Various therapies for these dental disorders have been established using artificial materials such as root canal treatments, scaling, and prosthesis procedures (Rosenstiel, 2001). After the loss of a tooth, the tooth functions are traditionally restored by replacement with an artificial tooth, the use of a bridge, and also osseo-integrated dental implants (Rosenstiel, 2001).

To restore the partial loss of organ functions and to repair damaged tissues, an attractive concept in regenerative therapy is stem cell transplantation into various tissues and organs (Grutner, 2008). The ultimate goal of regenerative therapy is to develop fully functioning bioengineered organs that can replace lost or damaged organs following disease, injury or aging (Ikeda & Tsuji, 2008; Purnell, 2008). It is expected that tooth replacement regenerative therapy will be established in the near future as a novel and successful biological treatment that will satisfy cosmetic, physiological and functional requirements. Recently, we reported fully functioning bioengineered tooth replacements after the transplantations of a bioengineered tooth germ or mature tooth unit comprising the bioengineered tooth and periodontal tissues such as periodontal ligament and alveolar bone into a lost tooth region (Nakao, 2007; Ikeda, 2009; Oshima, 2011). Tooth regenerative therapy has the potential to provide essential functional recovery and ultimately replace the current artificial materials used in dental treatments.

PRESENT STATUS OF OUR STUDY

Whole tooth regeneration approached from tooth organogenesis

The principle mechanisms of tooth organogenesis are regulated by reciprocal epithelial and mesenchymal interactions (Ikeda & Tsuji, 2008; Pispa &Thesleff, 2003). Recent studies of stem/progenitor cells and organogenesis have provided considerable new insights that have furthered our understanding of tooth tissue-derived stem cells. Dental tissue stem cells will have utility for the development of stem cell transplantation therapy to restore the partial loss of organ function and thereby achieve dental tissue repair such as caries and periodontal diseases (Mantesso & Sharpe, 2009). To generate whole tooth (Ikeda & Tsuji, 2008; Sharpe & Young, 2005), the approach is to recreate organogenesis through the epithelial-mesenchymal interactions that occur in the developing embryo and thereby develop fully functioning bioengineered organs from the resulting bioengineered organ germ generated via three-dimensional cell manipulation using immature stem cells *in vitro* (Fig. 4; Ikeda & Tsuji, 2008; Sharpe & Young, 2005). We thus

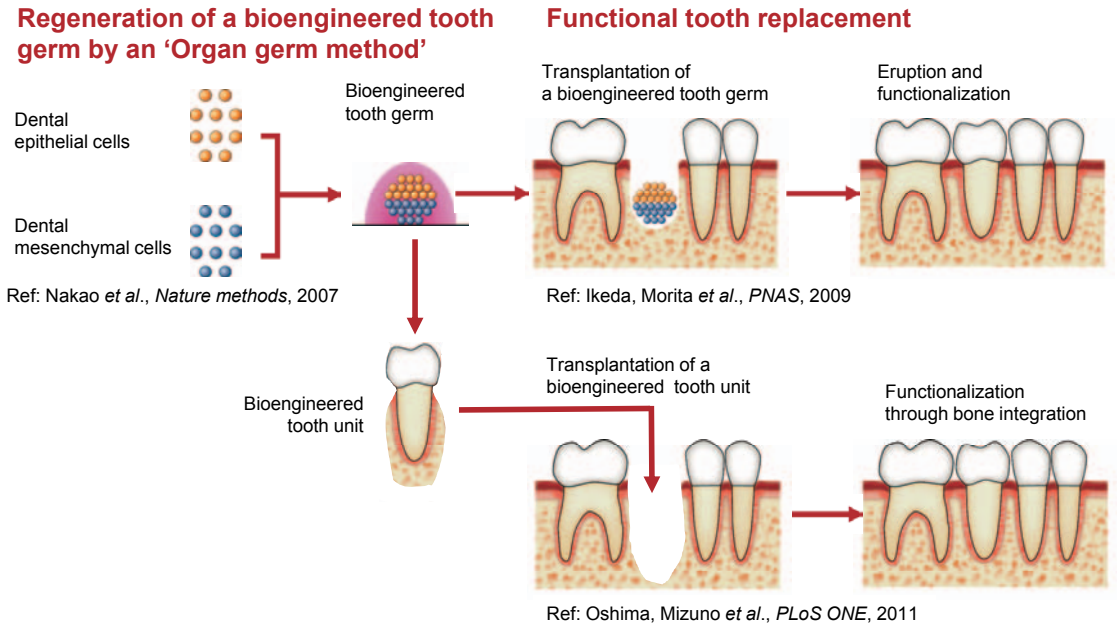

Figure 1. Strategies for whole tooth replacement via regenerative therapies
By transplanting bioengineered tooth germ reconstituted from epithelial and mesenchymal cells via the organ germ method, or transplanting bioengineered tooth units with periodontal ligament and alveolar bone developed from bioengineered tooth germ, functioning teeth are regenerated *in vivo*.

developed a bioengineering method for forming a three-dimensional organ germ in the early developmental stages, termed the 'bioengineered organ germ method' (Nakao, 2007). This method has the distinctive feature that the bioengineered tooth germ is reconstituted between cap-stage tooth-germ-derived epithelial and mesenchymal cells using cell compartmentalization at a high cell density in a collagen gel solution. The bioengineered tooth germs successfully developed into teeth with the correct tooth structures at high frequency (Figure 3). Direct cell-to-cell interactions induced by high cell density and cell compartmentalization are essential in tooth organogenesis. Thus, cell compartmentalization is thought to be effective for initiating organogenesis in a bio-engineered tooth germ.

Functional Tooth replacement by a transplantation of the bioengineered tooth germ

Teeth perform important oral functions, such as mastication, pronunciation, and facial aesthetics, which have an important influence on the quality of life as they facilitate both oral communication and nutritional intake (Proffit, 2007). These oral functions are achieved in harmony with the teeth, masticatory muscles and the temporomandibular joint under the control of the central nervous system. For the success of tooth replacement regenerative therapy, a bioengineered tooth must be capable of erupting in the lost tooth region in an adult oral environment and achieve full functionality, including sufficient masticatory performance, biochemical cooperation with the periodontal tissues and proper responsiveness to noxious stimulations via neurons in the maxillofacial region. The bioengineered molar tooth germ could erupt and reach occlusion with an opposing tooth at 49 days after transplantation in the mouse adult

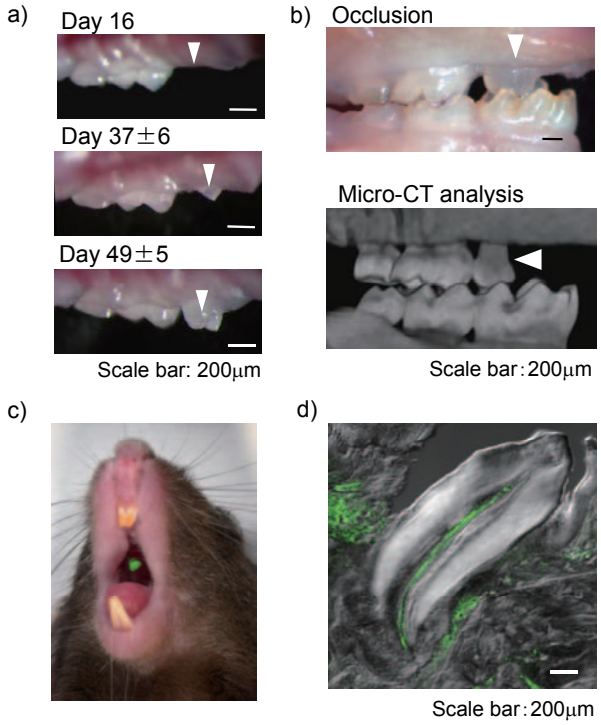

Figure 2. Functional tooth replacement by the transplantation of the bioengineered tooth germ
a) Eruption of a regenerated tooth after the transplantaion of a bioengineered tooth germ reconstituted by an organ germ method. b) Oral photograph (upper) and micro-CT analysis (lower) showing occlusion of the bioengineered tooth. c) Eruption of the GFP-labeled bioengineered tooth reconstituted between GFP-transgenic mice-derived epithelial and mesenchymal cells. d) Nerve fibers in a bioengineered tooth was analyzed by imminohistochemical analysis using anti-neurofilament-H antibody (Green).

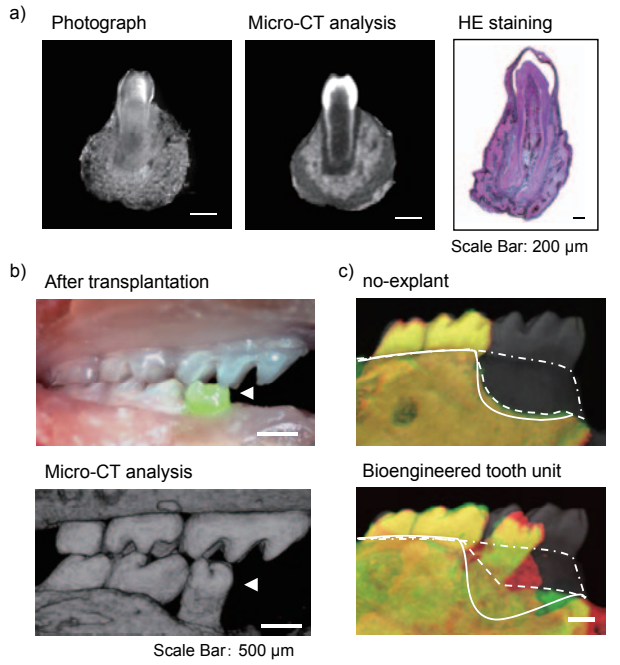

Figure 3. Functional tooth replacement by the transplantation of the bioengineered tooth unit
a) Oral photograph, micro-CT analysis and HE-staining showing the structure of a bioengineered tooth unit comprising a bioengineered tooth, periodontal ligament and alveolar bone. b) Transplantation of the bioengineered tooth unit (upper) and micro-CT analysis. c) Three-dimensional superposition of micro-CT images of natural dentition (gray, double dotted line), a transplanted bioengineered tooth unit (lower) and a no transplantation control (upper) at day 0 in an extensive bone defect (red, straight line), and at 45 days after transplantation (green, dotted line). The superior edges of the recipient alveolar bone are indicated by each line.

oral environment (Fig. 1A) (Ikeda, 2009). The periodontal ligaments of bioengineered teeth have been shown to successfully reproduce normal bone remodelling in response to mechanical stress, indicating that a bioengineered tooth can regenerate critical dental functions through the restoration and re-establishment of cooperation with the maxillofacial region. We have provided evidences that nerve fibres, such as the sensory and sympathetic nerves, innervating both the pulp and PDL of a regenerated tooth that had successfully erupted following the transplantation of bioengineered tooth germ, have the appropriate perceptive potential for nociceptive stimulations and can properly transduce these events to the central nervous system (Ikeda, 2009). Hence, whole tooth regenerative therapy involving the transplantation of the bioengineered tooth germ has the potential to fully restore tooth function

Functional tooth replacement by a transplantation of the bioengineered tooth unit

Transplantation of a bioengineered mature organ will lead to immediately perform of the full functions *in vivo* and have a profound impact on the survival outcomes of many diseases (Brockes & Kumar, 2005). In the dental treatment, it has been expected to transplant of a bioengineered tooth unit comprising mature tooth, periodontal ligament and alveolar bone into the tooth loss region through bone integration, which is connected between recipient bone and bioengineered alveolar bone in a bioengineered tooth unit. We thus investigated whether a bioengineered mature tooth replacement has the potential in the future dental regenerative therapy (Oshima, 2011). The bioengineered tooth unit, which was controlled for length and shape, was successfully transplanted into a properly-sized bony hole in the alveolar bone through bone integration by recipient bone remodeling in a murine transplantation model system. The bioengineered tooth unit restored enough the alveolar bone in a vertical direction into an extensive bone defect of murine lower jaw. Engrafted bioengineered tooth displayed physiological tooth functions such as mastication, periodontal ligament function for bone remodeling and responsiveness to noxious stimulations. This study thus represents a substantial advance and demonstrates the real potential for bioengineered mature organ replacement as a next generation regenerative therapy.

DISCUSSION

To realize the practical clinical application of tooth regenerative therapy, a major research hurdle is the identification of appropriate cell sources. Tooth regenerative therapy may be optimized by using the patient's own cells to avoid immunological rejection (Ikeda & Tsuji, 2008; Mantesso & Sharpe, 2009). Recent studies of stem cells and organogenesis have provided considerable advances in our understanding of the potential of stem cells as cell sources for regenerative therapies including the tooth tissue regenerative therapy (Mantesso & Sharpe, 2009). Candidate cell sources also include induced pluripotent stem cells (iPS cells).

Teeth have unique morphological features which are programmed at predetermined sites in the oral cavities during tooth development. Further studies are also required to establish bioengineering technologies to control tooth morphologies, including tissue engineering using scaffolds, the identity of morphogenesis-related genes and the appropriate cytokines to use during the course of morphogenesis. Tooth regenerative therapy is now regarded as a crucial study model for future replacement regenerative therapies for other more combplex organs and will contribute substantially to the required understanding to regenerate various organs.

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REFERENCES

Brockes, J.P. and Kumar, A. (2005). Appendage regeneration in adult vertebrates and implications for regenerative medicine. *Science* 310: 1919-1923.

Gurtner, G.C., *et al*. (2008). Wound repair and regeneration. *Nature* 453: 314-321.

Ikeda, E. and Tsuji, T. (2008). Growing bioengineered teeth from single cells: potential for dental regenerative medicine. *Expert Opin Biol Ther* 8: 735-744.

Ikeda, E., *et al*. (2009). Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci U S A* 106: 13475-13480.

Mantesso, A. and Sharpe, P. (2009). Dental stem cells for tooth regeneration and repair. *Expert Opin Biol Ther* 9: 1143-1154.

Nakao, K., et al. (2007). The development of a bioengineered organ germ method. *Nat Methods* 4: 227-230.

Oshima, M. *et al*. (2011). Functional Tooth Regeneration Using a Bioengineered Tooth Unit as a Mature Organ Replacement Regenerative Therapy, PLoS *ONE* 6: e21531.

Pispa, J. and Thesleff, I. (2003). Mechanisms of ectodermal organogenesis. *Dev Biol* 262: 195-205.

Proffit, W.R., *et al*. (2007). *Contemporary orthodontics*. Mosby Elsevier.

Purnell, B. (2008). New release: the complete guide to organ repair. Introduction. *Science* 322: 1489.

Rosenstiel, S.F., *et al*. (2001). *Contemporary fixed prosthodontics*. Mosby.

Sharpe, P.T. and Young, C.S. (2005). Test-tube teeth. *Sci Am* 293: 34-41.

Gingiva-derived iPS cells: A promising tool for dental applications

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Key words: Dentistry, Fibroblasts, Gingival, iPS cell, Reprogramming, Tissue engineering

ABSTRACT

Induced pluripotent stem (iPS) cells can be generated through the reprogramming of somatic cells from different tissues by forced expression of defined exogenous factors. These iPS cells efficiently generated from accessible tissues have the potential to be used for various clinical applications. The oral gingiva is an easily obtainable tissue for dentists, and cells can be isolated from patients with minimal discomfort. We successfully generated iPS cells from adult mouse or human gingival fibroblasts (GFs) via transduction of the Yamanaka factors (Egusa et al., 2010). GFs demonstrate a higher reprogramming efficiency than the skin fibroblasts which have been conventionally used for the generation of iPS cells. The generation of iPS cells from the gingiva is expected to provide a breakthrough in the dental sciences because it offers a promising method for the easy production of pluripotent stem cells by dental researchers, for oral tissue regeneration, as well as for *in vitro* applications for drug screening and the generation of disease-specific iPS cells for tailor-made diagnostics. In this review, I first address the advantage of GFs as an iPS cell source, followed by a discussion of the latest findings and future challenges regarding iPS cell research in the field of dentistry.

INTRODUCTION

The induction of pluripotency by transcription factors (e.g., Oct3/4, Sox2, Klf4 and c-Myc) has now become a commonplace method to produce pluripotent stem cells. These cells, known as induced pluripotent stem (iPS) cells, are considered an attractive alternative to embryonic stem (ES) cells as they do not require the destruction of human embryos, and theoretically avoid problems with rejection following implantation of non-autologous cells (Takahashi et al., 2007). Therefore, iPS cells are expected to be useful for many applications, including disease modeling, autologous cell therapy, drug or toxicity screening and basic research.

This review describes the generation of iPS cells from gingival fibroblasts (GFs) and the possible applications of these iPS cells in the field of dentistry. The advantages of GFs compared to other dental tissue cells as an iPS cell source is also discussed, and the current hurdles that will need to be cleared if iPS cells are to fulfill their clinical promise are also outlined in this review.

GFs AS AN iPS CELL SOURCE

Characteristics of GFs

The gingiva is composed of a thin keratinocyte layer with underlying highly-vascularized connective tissue. GFs, which are the main constituents of the gingival connective tissue, are phenotypically different from other fibroblasts (Stephens et al., 2001). Interestingly, clinical observations and experimental animal studies consistently show that wound healing in the oral mucosa has better outcomes than that in the skin (Sciubba et al., 1978), although the healing processes and sequences are similar. GFs play an important role in oral mucosal wound healing, and it has been postulated that these cells contribute to the favorable early wound closure by expressing distinctive genes (Sukotjo et al., 2003). In addition, GFs adhere and spread well on culture plates, and proliferate well under relatively simple culture conditions (Giannopoulou and Cimasoni, 1996). It is conceivable that these characteristics of GFs would favorably affect their reprogramming efficiency, and GFs also have an advantage in the establishment of primary cell cultures with regard to their accessibility and ease of culture.

Generation of iPS Cells from GFs

The gingiva is frequently resected during general dental treatments, and it has traditionally been treated as biomedical waste. We generated iPS cells from human GFs, established from healthy gingival tissues discarded during dental implant surgery, using retroviral transduction of the Yamanaka factors (Oct3/4, Sox2, Klf4 and c-Myc) (Fig. 1; upper panels) (Egusa et al., 2010). It should be noted that the proto-oncogene product c-Myc markedly accelerates the reprogramming process (Nakagawa et al., 2008), but also increases tumor formation in iPS cell-derived chimeric mice, thereby hindering potential clinical applications. We also successfully generated iPS cells from adult wild-type mouse GFs via introduction of three factors; i.e. the same Yamanaka factors, but without the c-Myc oncogene.

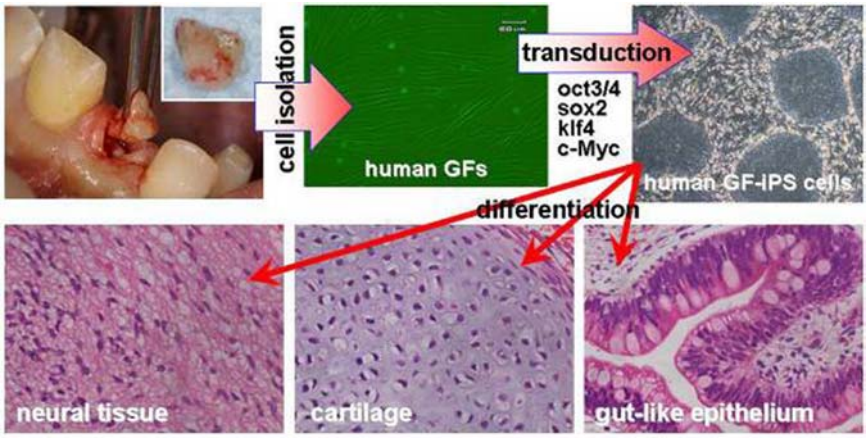

Fig. 1. The generation of human GF-derived iPS cells.
The patient's gingiva resected during dental implant surgery was used to establish the primary GF culture. After four-factor retroviral transduction, several ES cell-like colonies emerged and were picked-up for clonal iPS cell cultures (upper panels). H&E staining of teratoma sections showed differentiation of the human GF-iPS cells into various tissues from all germ layers, including the ectodermal neural tissue, mesodermal cartilage and endodermal gut-like epithelium (lower panels). The figure was reproduced under the open-access license policies of the Public Library of Science (PLoS), taken from PLoS One (Egusa et al., 2010).

Table 1. The reprogramming efficiency of somatic cells from different tissues

Authors (Year)	iPS cell source	Reprogramming method	Efficiency	Time
Takahashi *et al*. (2007)	Human skin fibroblasts	O/S/K/M (RV)	0.02%	25-30 days
Nakagawa *et al*. (2008)	Mouse tail-tip skin fibroblasts	O/S/K (RV)	0.005%	30 days
Kim *et al*. (2008)	Mouse neural stem cells	O/S/K/M (RV)	3.6%	21 days
Aa. (2sen *et al*008)	Human keratinocytes	O/S/K (RV)	<0.06%	20 days
		O/S/K/M (RV)	<1%	14-21 days
Tsai *et al*. (2010)	Mouse dermal papilla cells	O/S/K/M (RV)	1.38%	10 days
Sun *et al*. (2009)	Human adipose stem cells	O/S/K/M (LV)	<0.2%	16 days
Yan *et al*. (2009)	SHED	O/S/N/L (LV)	<0.08%	21 days
	Human SCAP	O/S/N/L (LV)	0.07%	21 days
	Human dental pulp stem cells	O/S/K/M (RV)	0.1%	21 days
Tamaoki *et al*. (2010)	Human dental pulp cells	O/S/K (RV)	<0.06%	30 days
		O/S/K/M (RV)	<0.24%	21 days
Oda *et al*. (2010)	Human dental papilla cells	O/S/K (RV)	<0.03%	30 days
Miyoshi *et al*. (2010)	Human buccal mucosa fibroblasts	O/S/K/M (RV)	0.02%	13-25 days
Egusa *et al*. (2010)	Mouse gingival fibroblasts	O/S/K (RV)	<0.02%*	30-50 days*
		O/S/K/M (RV)	<1.2%	14-21 days

Reprogramming factors (O: Oct3/4, S: Sox2, K: Klf4, M: c-Myc, N: Nanog, L: Lin28), Viral vector (RV: retrovirus, LV: lentivirus, SV: sendai virus), SHED: Stem cells from human exfoliated deciduous teeth, SCAP: Stem cells from apical papilla, *Unpublished data

These iPS cells exhibited the morphology and growth properties of ES cells and expressed ES cell marker genes, with a decreased CpG methylation ratio in the promoter regions of Nanog and Oct3/4. Additionally, teratoma formation assays showed the ES cell-like derivation of cells and tissues representative of all three germ layers (Fig. 1; lower panels). When transplanted into blastocysts, the mouse GF-derived iPS cells generated by the three-factor transduction gave rise to chimeras and contributed to the development of the germline. These results suggest that high-quality iPS cells can be generated from adult mouse GFs by transduction of the three factors without any specific system for the selection of reprogrammed cells.

Reprogramming Efficiency

The reprogramming process is likely affected by many factors, including the type and origin of the cells used. Many types of somatic cells from different tissues from all three germ layers have been used for iPS cell generation (Table 1). In the field of dentistry, iPS cells have been generated from a variety of oral mesenchymal cells, such as apical papilla stem cells (Yan et al., 2010a), dental pulp cells (Tamaoki et al., 2010; Yan et al., 2010a), dental papilla mesenchymal cells (Oda et al., 2010), buccal mucosa fibroblasts (Miyoshi et al., 2010), GFs (Egusa et al., 2010) and periodontal ligament fibroblasts (Wada et al., 2011). Among the somatic cells, some cell types such as keratinocytes (Aasen et al., 2008), dermal papilla cells (Tsai et al., 2010) and tissue stem cells (Kim et al.,

2008; Sun et al., 2009; Tamaoki et al., 2010) demonstrated a high reprogramming efficiency associated with their high expression of endogenous reprogramming factors and/or ES cell-associated genes, thus suggesting that these cells have epigenetic advantages for reprogramming.

For clinical applications, iPS cells that can be efficiently generated from easily accessible tissues have great potential. Interestingly, although GFs do not highly express endogenous reprogramming factor genes, they show a relatively high reprogramming efficiency, possibly because of the high proliferation rate of the GFs supported by the high expression of the telomelase-activity enhancer gene, Tert (Egusa et al., 2010). Although the reprogramming efficiency of human GFs is higher than that of human skin fibroblasts (Egusa et al., 2010) or periodontal ligament fibroblasts (Wada et al., 2011), it may be inferior to undifferentiated stem cells, such as dental stem cells. Nonetheless, GFs represent an ideal autologous cell source allowing for easy collection of a large number of cells by dentists that can be grown in a simple culture system, and that can quickly be cultured into quantities sufficient to obviate extensive and long-term expansion. Therefore, the production of iPS cells from GFs is expected to be a useful technology for clinical applications in dentistry, although there are still many hurdles that need to be overcome, as described in the next section, if iPS cells are to fulfill their clinical promise.

PROBLEMS ASSOCIATED WITH THE CLINICAL APPLICATION OF iPS CELLS

The iPS cell technology is at the cutting edge of scientific research, and major progress has been achieved; however, the applicability of these cells in clinical trials still has to be carefully investigated. The major drawback of using the iPS cells for clinical applications is the possibility of tumor formation after their *in vivo* implantation over the lifespan of the implant or the patient.

To avoid this concern, reduced genetic manipulation without using viral vectors needs to be considered for gene insertion into somatic cells in order to prevent insertional mutagenesis. In this regard, the generation of iPS cells by delivery of reprogramming proteins (Kim et al., 2009) or microRNAs (Miyoshi et al., 2011) holds significant potential; however, the reprogramming efficiency of these methods is currently impractically low and needs to be improved. Recently, an efficient method to generate genome integration-free iPS cells using episomal plasmid vectors was reported (Okita et al., 2011), and we revealed that this method enables human GFs to be easily reprogrammed into iPS cells (Egusa H. et al. unpublished data).

It is also important to establish xeno-free culture methods for human iPS cell culture before such cells can be made available for clinical transplant applications. Many trials to generate iPS cells using xeno-free culture methods are underway, such as serum-free defined conditions (Hayashi et al., 2010) and animal feeder cell-free autologous culture (Takahashi et al., 2009). We recently revealed that parental human GFs, used as an alternative to the conventionally used mouse-derived feeder cells, can maintain the undifferentiated status of GF-derived iPS cells, thus suggesting that autologous GFs represent not only a source for iPS cells, but also for feeder layers (Egusa H. et al. unpublished data).

Another problem to be solved is how to direct cell differentiation toward a specific cell fate or tissue type in order to avoid tumor formation in the transplanted site. Several approaches, such as cell sorting via lineage-specific markers, have been tried to address this critical issue; however, a simpler alternative method may be required, because such sorting is likely to increase the risk of cell loss and contamination. We are currently using a mouse model to investigate whether the application of small molecules with potent osteogenic-inducing activity can distinctively guide GF-derived iPS cells into osteogenic cells to achieve robust osteogenesis after their transplantation.

THE FUTURE DIRECTION OF iPS CELL TECHNOLOGY IN DENTAL SCIENCE

GF-Derived iPS Cells for Oral Tissue Engineering

One of the major anticipated applications of iPS cells for both the medical and dental fields is for regenerative medicine. In dentistry, GF-derived iPS cells hold great potential for oral tissue regeneration, such as for periodontal tissues, jaw bones and salivary glands. In a mouse model, iPS cells combined with enamel matrix derivatives greatly enhanced periodontal regeneration by promoting the formation of cementum, alveolar bone and a normal periodontal ligament (Duan et al., 2011). We are currently investigating whether the stable osteogenic capacity of mouse GF-derived iPS cells can be exploited by fabricating cells in three-dimensional constructs, which would be useful for implanting into a bone defect as a cell-based bone graft material.

The GF-derived iPS cells are also considered to be a promising stem cell source for tooth regeneration. The use of a patient's own dental tissue-derived stem cells has serious limitations because it would require the extraction of a remaining tooth. Tooth bioengineering will therefore likely require the use of reprogrammed stem cells, and the GF-derived iPS cell technology may open up the possibility of using autologous GFs for tooth bioengineering purposes.

GF-Derived iPS Cell Lines for Cell Banking

A great advantage of human iPS cells is their unlimited proliferation without transformation toward carcinogenic cells, which could enable cell banking to produce quality-controlled differentiated cells in sufficient quantities for mass production to be used for the treatment of multiple patients. It should be noted that dental pulp cell lines from 107 individuals have been established at Gifu University in Japan, and are considered to be a promising source of iPS cells banked for use in regenerative medicine (Tamaoki et al., 2010). In addition, there are 2 donated dental pulp cell samples in their collections that were homozygous for 3 HLA-loci (HLA-A, B, DR), which are considered to be highly valuable for predicting the intensity of immunological rejection after transplantation therapy. On the other hand, it is estimated that approximately 140 donors with the unique HLA homozygous haplotypes would be needed to cover 90% of the Japanese pop-

ulation with a perfect match, and it would be necessary to type ~160,000 individuals to collect these haplotypes (Okita et al., 2011). In order to collect such a large number of cell lines from each individual, GFs, which are more easily accessible than dental pulp cells and can be readily reprogrammed into iPS cells, could be a promising candidate for establishment of an iPS cell bank in dentistry. The HLA-homozygous GF-derived iPS cells may provide a useful allograft therapeutic strategy for future applications in regenerative medicine.

GF-Derived iPS Cells for *in vitro* Applications

Other possible applications of patient-specific iPS cells in dentistry would be *in vitro* use, such as for oral and craniofacial disease modeling and the development of pharmacological agents for alveolar bone augmentation, oral cancer treatment and so on. These *in vitro* applications of patient-specific iPS cells from GFs may be a realistic possibility, because the isolation and growth of the cells is not associated with patient safety or ethical issues as would be the case for the transplantation of iPS cells for regenerative medicine. iPS cells as a dental-associated disease model have already been established from dental stem cells obtained from a patient with oculofacio-cardio-dental (OFCD) syndrome, a development disorder (Yan et al., 2010b). These iPS cells can be utilized to study the aberrant regulation of gene activation during the process of *in vitro* cell differentiation resulting from the BCL-6 co-repressor mutation, which is responsible for this syndrome.

CONCLUSIONS

In summary, GFs are easily obtained by dentists, and can be readily reprogrammed into iPS cells, thus making them a promising cell source for future oral tissue engineering applications, as well as for *in vitro* applications for drug screening and the generation of disease-specific iPS cells for tailor-made diagnostics. The GF-derived iPS cell technology allows for easy generation and handling of iPS cells for dental researchers, and would represent a major step to open up new avenues for dental research and personalized treatment.

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REFERENCES

Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, et al. (2008). Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 26 (11): 1276-84.

Duan X, Tu Q, Zhang J, Ye J, Sommer C, Mostoslavsky G, et al. (2011). Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. *J Cell Physiol* 226 (1): 150-157.

Egusa H, Okita K, Kayashima H, Yu G, Fukuyasu S, Saeki M, et al. (2010). Gingival fibroblasts as a promising source of induced pluripotent stem cells. *PLoS One* 5 (9): e12743.

Giannopoulou C, Cimasoni G. (1996). Functional characteristics of gingival and periodontal ligament fibroblasts. *J Dent Res* 75 (3): 895-902.

Hayashi Y, Chan T, Warashina M, Fukuda M, Ariizumi T, Okabayashi K, et al. (2010). Reduction of N-glycolylneuraminic acid in human induced pluripotent stem cells generated or cultured under feeder- and serum-free defined conditions. *PLoS One* 5 (11): e14099.

Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, et al. (2009). Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 4 (6): 472-6.

Kim JB, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, et al. (2008). Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 454 (7204): 646-50.

Miyoshi K, Tsuji D, Kudoh K, Satomura K, Muto T, Itoh K, et al. (2010). Generation of human induced pluripotent stem cells from oral mucosa. *J Biosci Bioeng* 110 (3): 345-50.

Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, et al. (2011). Reprogramming of Mouse and Human Cells to Pluripotency Using Mature MicroRNAs. *Cell Stem Cell* 8 (6): 633-8.

Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 26 (1): 101-6.

Oda Y, Yoshimura Y, Ohnishi H, Tadokoro M, Katsube Y, Sasao M, et al. (2010). Induction of pluripotent stem cells from human third molar mesenchymal stromal cells. *J Biol Chem* 285 (38): 29270-8.

Okita K, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, et al. (2011). A more efficient method to generate integration-free human iPS cells. *Nat Methods* 8 (5): 409-12.

Sciubba JJ, Waterhouse JP, Meyer J. (1978). A fine structural comparison of the healing of incisional wounds of mucosa and skin. *J Oral Pathol* 7 (4): 214-27.

Stephens P, Davies KJ, Occleston N, Pleass RD, Kon C, Daniels J, et al. (2001). Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix reorganization and matrix metalloproteinase activity. *Br J Dermatol* 144 (2): 229-37.

Sukotjo C, Lin A, Song K, Ogawa T, Wu B, Nishimura I. (2003). Oral fibroblast expression of wound-inducible transcript 3.0 (wit3.0) accelerates the collagen gel contraction in vitro. *J Biol Chem* 278 (51): 51527-34.

Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, et al. (2009). Feeder-free derivation of induced pluripo-

tent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A* 106 (37): 15720-5.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131 (5): 861-72.

Takahashi K, Narita M, Yokura M, Ichisaka T, Yamanaka S. (2009). Human induced pluripotent stem cells on autologous feeders. *PLoS One* 4 (12): e8067.

Tamaoki N, Takahashi K, Tanaka T, Ichisaka T, Aoki H, Takeda-Kawaguchi T, et al. (2010). Dental pulp cells for induced pluripotent stem cell banking. *J Dent Res* 89 (8): 773-8.

Tsai SY, Clavel C, Kim S, Ang YS, Grisanti L, Lee DF, et al. (2010). Oct4 and Klf4 Reprogram Dermal Papilla Cells into Induced Pluripotent Stem Cells. *Stem Cells* 28 (2): 221-228.

Wada N, Wang B, Lin NH, Laslett AL, Gronthos S, Bartold PM. (2011). Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts. *J Periodontal Res* 46 (4): 438-47.

Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT. (2010a). iPS cells reprogrammed from mesenchymal-like stem/progenitor cells of dental tissue origin. *Stem Cells Dev* 19 (4): 469-80.

Yan X, Qin H, Shi S, Huang G. (2010b). Human iPS cells reprogrammed from stem cells with BCOR mutation. *J Dent Res* 89 (Sp. Iss. B): 2048.

Poster Session

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01-1

Interleukin-8 and Interleukin-10 expression on traumatic ulcer treated with lactoferrin 10mg/ml

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BACKGROUND : Traumatic ulcer is a disturbance in oral cavity with a high prevalence worldwide. This condition relates to alterations in cytokine expression. Until now, treatment is focused on symptomatic relief using medicaments made of chemical substances. As we know, chemical substance is not always safe and often has a side effect. It is necessary to find an alternative treatment using natural substance possessing immunomodulating ability. Lactoferrin, a protein derived from bovine milk, has an ability to modulate cytokine production and activate macrophages directly. During inflammation, Lactoferrin inhibit the effector phases in cellular immune response.

PURPOSES : This proposed study aims to determine the role of Lactoferrin in the expression of IL-8 and IL-10 in traumatic ulcer.

METHODS : A post only control group design study will be performed on 21 mice, divided evenly and randomly into 3 groups. Traumatic ulcer will be created on Group 1 and 2 using heated burnisher. Group 1 will receive topical Lactoferrin gel treatment. Group 2 will receive topical Povidon Iodine treatment. Group 3, serving as control, will not be wounded and treated with any medicament. Expression of IL-8 and IL-10 is detected using immunohistochemistry using specific monoclonal antibody.

CONCLUSION : This proposed study will provide the basis of a new approach in the therapy of traumatic ulcer.

Key words : lactoferrin, interleukin-8, interleukin-10, expression, traumatic ulcer

01-2

Actinobacillus actinomycetemcomitans fimbrial adhesin profile from patients with aggressive periodontitis in Clinic of Periodontic Faculty of Dentistry Airlangga University

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BACKGROUND : Actinobacillus actinomycetemcomitans is regarded as one of the key bacterial agents associated with aggressive periodontitis in young adult and an important factor in pathogenesis bacteria, as it acts as a primary etiologic agent in this disease. Actinobacillus actinomycetemcomitans is strongly associated with progressing periodontitis in young adult patients and caused severe also rapid form of periodontal disease that affect adolescent. Actinobacillus actinomycetemcomitans possesses a large number of virulence factors with a wide range of activities and also interfere with tissue repair i.e fimbriae. The mechanisms virulence of Actinobacillus actinomycetemcomitans are adhesion, invasion of epithelial cells, colony phase variation, interference with host defense mechanisms, bone resorption and apoptosis. If a bacterium cannot adhere to a particular environment, it cannot survive. Actinobacillus actinomycetemcomitans possesses fimbriae with a protein that has a subunit of molecular mass 54kDa in transparent rough colony.

PURPOSE : The aim of the study was to determine Actinobacillus actinomycetemcomitans fimbrial adhesin profile from patients with aggressive periodontitis in Clinic of Periodontic Faculty of Dentistry Airlangga University.

METHOD : Subgingival plaque were taken from patients with aggressive periodontitis. AAGM used for clinical isolated Actinobacillus actinomycetemcomitans with added 75ug/ml Bacitracin and 5ug/ml Vancomycin. Actinobacillus actinomycetemcomitans isolated from subgingival pocket more than 5mm in patients with aggressive periodontitis. Samples of subgingival plaque were taken using sterilized paper point introduced into periodontal pocket for 60second, was incubated in anaerobic condition at 37°C, for 4-5 days. The omnimixer modification was used to cut Actinobacillus actinomycetemcomitans fimbriae for three times and every times was 15minutes. Hemagglutination and SDS-PAGE test on fimbriae fractions considering that hemagglutinin protein can also function as an adhesin protein.

RESULT : The hemagglutinin protein fimbriae resulted had a molecular weight of about 53,3kDa at SDS-PAGE.

CONCLUSION : The 53.3kDa protein fimbriae is an adhesin candidate from Actinobacillus actinomycetemcomitans isolate Surabaya which is a material choice to manage patient with aggressive periodontitis.

Key words : Actinobacillus actinomycetemcomitans, fimbrial adhesin, aggressive periodontitis,

01-3

Variant analysis of HLA-DRB1 on immunogenetic pathway of sIgA secretion in saliva as dental caries risk (Study in javanesse population in Surabaya-Indonesia)

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HLA-DRB1 allele was derived from MHC class II molecules and play an important role in the control of antigen peptides presentation to TCR that encoded sIgA secretion which contribute to prevent S. mutans colonization.

The purpose of this study were to determine the role of HLA-DRB1 polymorphisms that affect the CD4 count and TGF-β1 expression on immunogenetic pathway of sIgA secretion in saliva and raises the risk of dental caries.

Methods of this research were DNA analysis on case and control sample groups with low and high levels of sIgA by ELISA test. HLA-DRB1 polymorphisms were studied through DNA isolation for PCR-RFLP proceeded with sequencing. Immunogenetic pathway of sIgA secretion was studied through the relationship between HLA-DRB1 variant to CD4 count and TGF-β1 expression.

Results proved that nomenclature of HLA-DRB1 variant DRB * 1209 (2), DRB * 1209 (3) and DRB * 1209 (4) were associated with low sIgA levels and high caries risk. Whereas DRB * 1209 and DRB * 1209(1) were associated with high sIgA levels and low caries risk. Path analysis showed that CD4 affects sIgA via TGF-β1, because TGF-β1 directly influence the sIgA and CD4 effect on TGF-β1.

The study has concluded that mutation in HLA-DRB1 alleles cause changes in sIgA levels and therefore contributes to the risk of dental caries. HLA-DRB1 polymorphisms contributes to the change in CD4 cell count and changes in expression of TGF-β1 on the immunogenetic pathway of sIgA secretion.

Key words : HLA-DRB1, DNA analysis, sIgA level, CD4 count, TGF-β1 expression, dental caries.

02-1

IL-6 induced Rex-1 expression in stem cells from human exfoliated deciduous teeth

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INTRODUCTION : Interleukin-6 (IL-6) is a multifunctional cytokines that regulates immune response, inflammation, hematopoiesis and oncogenesis. Previously, we found that mechanical stress induced IL-6 release in SHED (stem cells from human exfoliated deciduous teeth). However, little is known about the roles of IL-6 in SHED.

OBJECTIVES : The aim of this study was to investigate the effects of IL-6 on the expression of Rex-1 in SHED.

MATERIALS AND METHODS : Cultures of SHED was established from the explants of pulp tissue. Cells were stimulated with 0-5ng/ml of IL-6 in serum-free condition for 2 hours. Expression of Rex-1 was examined by quantitative Reverse Transcription- Polymerase Chain Reaction (qRT- PCR) and immunocytochemistry. The molecular mechanism of IL-6-induced Rex-1 expression was investigated by means of inhibitors.

RESULTS : Application of exogenous IL-6 in the culture could increase Rex-1 mRNA in a dose dependent manner. The inductive effect of IL-6 on Rex-1 expression was further confirmed by immunocytochemistry analysis. IL-6-induced Rex-1 expression could be inhibited by specific P2Y1 and P2Y6 antagonist, suggesting the role of nucleotides in IL-6- induced Rex-1 expression. The role of nucleotides was supported by the induction effect of exogenous ATP and UDP on Rex-1 expression.

CONCLUSIONS : IL-6 could up-regulate Rex-1 expression via induction of nucleotides release. Since Rex-1 is one of the embryonic stem cells markers, induction of Rex-1 might support the stemness of SHED. This finding leads to the possibility that IL-6 might be one of the factors that provide the suitable niche for SHED.

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02-2

Stress-induced IL-1β release in HPDL via vesicles and P2X7R/Pannexin-1 complex

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INTRODUCTION : Mechanical stress is one of the factors involved in the homeostasis of periodontium. Previous studies showed mechanical stress induced the release of ATP and cytokines in human periodontal ligament (HPDL) cells.

OBJECTIVE : The aim of this study was to examine the mechanism of mechanical stress on the release of inflammatory cytokines.

METHODS : HPDL cells were subjected to compressive stress by direct loading of weight on the culture. The expression and release of IL-1β were examined by reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. The molecular mechanism of stress-induced cytokine expression was investigated by means of inhibitors.

RESULTS : Mechanical stress induced expression of IL-1β mRNA and proteins in HPDL cells, when compared to the control in a force dependent manner. The induction was inhibited by probenecid (pannexin-1 hemichannel inhibitor) and KN-62 (P2X7 receptor antagonist), suggesting the involvement of hemichannel gap junction and P2/nucleotide receptors. Interestingly, addition of vesicular trafficking inhibitors, monensins, N-ethylmaleimide (NEM) and brefeldin-A significantly attenuated the release of both ATP and IL-1β. Immunocytochemistry approach revealed the co-localization of Pannexin-1 and P2X7 receptor on the membrane of HPDL cells. Moreover, the membrane translocation of SNAP-25, a core protein of vesicular-membrane fusion SNARE complex, after stress treatment and co-localization of SNAP-25 with P2X7 receptor were observed.

CONCLUSIONS : The results from this study indicated that mechanical stress could directly regulate the expression of IL-1β in HPDL cells. The induction involved the function of pannexin-1 and P2X7 receptor. The release of IL-1β was also depended on the vesicular trafficking. It is possible that Pannexin-1, in combination with P2X7 receptor, could function in docking the vesicular release of IL-1β upon mechanical stress activation in HPDL cells. Taken together, our results raised the possibility of the novel function of hemichannels as a docking site for vesicular release.

02-3

Estradiol-induced osteoprotegerin in dental pulp cells via surface estrogen receptor

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INTRODUCTION : Estrogen plays an important role in bone remodeling. Evidences from ovarectomized rat also indicated that estrogen affect the rate of dentin formation. Moreover, it has been shown that both odontoblast and dental pulp cells expressed estrogen receptor.

OBJECTIVE : The aim of this study was to investigate the effect of 17beta estradiol in human dental pulp cells, especially on the expression of receptor activator of nuclear factor kB ligans (RANKL) and osteoprotegerin (OPG).

METHODS : Cultured of human dental pulp cells was treated with 10^{-5} to 10^{-8} M of 17beta estradiol for 24 hours. The cell number was determined by MTT assay. Reverse transcription polymerase chain reaction was used to determine the pattern of gene expression. Western analysis or ELISA was used to confirm the change in protein level.

RESULTS : The results showed that estradiol could reduce RANKL/OPG expression ratio both in transcription and translation levels. Up-regulation of OPG also observed when cell were treated with BSA conjugated estradiol indicated that the signal was generated from cell surface. Application of ERK inhibitor could suppress the inductive effect of estrogen, indicating the involvement of ERK signaling pathway.

CONCLUSIONS : In conclusion, this study indicated that human dental pulp cells responded to 17 beta estradiol by increasing the expression and synthesis of OPG. The signaling pathway was possibly occurred at the cell surface, indicating the presence of estrogen cell surface receptor and involved the ERK signaling pathway. The results support the importance of estrogen in dental pulp homeostasis.

03-1

Prevalence of oral herpes patients in dental clinic of the Faculty of Odonto-Stomatology from 2005 to 2009

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BACKGROUND : Oral herpes is an infection caused by the Herpes Simplex Virus type 1. The virus causes painful sores on the lips, gums, tongue, roof of your mouth, and inside your cheeks.

OBJECTIVE : The aim of this study was to demonstrate the prevalence of patients with oral herpetic infection in dental clinic of the Faculty of Odonto-Stomatology from 2005 to 2009. All patients from the young to the old ones live both in the city and countryside of Cambodia.

METHODS : 1,273 patients were involved in this cross sectional study, taking place from January in 2005 to December in 2009. After writing consent form, all the patients were asked by following WHO dental examination chat. The patients were not urged to come here; therefore, those were the acceptable samples. The data were recorded carefully and thoroughly in Department of Oral Pathology and computerized by using STATA version 10.

RESULTS :

Years	2005	2006	2007	2008	2009
Patients	254 (19.95%)	271 (21.29%)	259 (20.35%)	262 (20.58%)	227 (17.83%)

Year Groups	5-9	10-17	18-29	30-52
Patients	363 (28.51%)	598 (46.98%)	189 (14.85%)	123 (9.66%)

CONCLUSION : According to this cross sectional study, we found that adult patients were attacked noticeably due to kissing, sharing food, drink, towels, and dental implements. Also, the seroprevalence of the old patients was higher than the young. It meant that they had high immunity system against Herpes Simplex Virus type 1.

04-1

The efficiency of milk and egg white in storage of avulsed teeth.

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BACKGROUND AND RATIONALE : Avulsion- a complete displacement of a tooth from its socket, which is one of the most severe forms of dental trauma, demands of the tooth to be replanted without delay after the injury for a good prognosis. If immediate reimplantation cannot be accomplished, the tooth should be stored in a proper conserving media in an effort to preserve the viability of the periodontal ligament (PDL) cells. However, some commercial storage media are quite expensive and rarely available in emergency situations.

STUDY OBJECTIVE : The purpose of this study was to determine the ability of available media in Vietnam (pasteurized milk and egg white) compared to Hank's Balanced Salt Solution (HBSS- cell preserving solutions) in maintaining the viability of human PDL cells on avulsed teeth.

DESIGN AND EXPERIMENTAL METHODS USED : 45 healthy permanent teeth freshly extracted were randomly divided into 9 groups (5 teeth per group). Following extraction, the teeth were instantly immerged in 10ml media (HBSS, milk, egg white) during 30, 90 and 240 minutes at room temperature. PDL cells were released using enzymatic digestion procedure by dispase/collagenase. After staining with trypan blue, viable and non-viable cells were counted using a hemocytometer and converted to percentages for statistical comparison.

ESSENTIAL RESULTS : Non-parametric analysis indicated that egg white took a better preserving effect on cellular vitality than HBSS and milk at any time, especially at 90 minutes, egg white performed significantly a difference with p=0.01. In a same media, there was a reduction statistically in the percentages of viable cells with time (30, 90 and 240 minutes) for all protocols ($P \leq 0.05$).

CONCLUSION : We suggest that egg white, which is popular and affordable, is an effective storage media for avulsed teeth. To warrant the viability of teeth's PDL after traumas, the ideal conserving extra-alveolar duration is within 30 minutes.

05-1

Systems biology unravels a novel pathogenic mechanism of heterogeneous *P. gingivalis* lipid A structures in chronic periodontitis

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BACK GROUND : *Porphyromonas gingivalis* lipopolysaccharide (PgLPS) is a crucial virulence factor strongly associated with chronic periodontitis. *P. gingivalis* exploits its ability to shift between tetra-acylated ($LPS_{1435/1449}$) and penta-acylated (LPS_{1690}) lipid A isoforms during periodontitis.

OBJECTIVES : Present study was designed to comprehensively investigate the modulation of immuno-inflammatory activity, lipid perioxidation and apoptotic pathways in human gingival fibroblasts (HGFs) by PgLPS isoforms.

EXPERIMENTAL METHODS : Primary HGFs were treated with P. *gingivalis* $LPS_{1435/1449}$ and LPS_{1690} in dose-dependent and time-dependent experiments. Involvement of TLRs and signal transduction pathways were examined using blocking assays. Down-stream markers were examined by qPCR, ELISA and Western blot. Next, systems biology tools i.e. proteomics, metabolomics, transcriptomic and bioinformatics were used to unravel the global protein and gene expression of HGF for PgLPS isoforms. Generic and specific biomarkers were cataloged using bioinformatics tools. RNA harvested from HGFs was subjected to transcriptomic analysis using gene-arrays in Toll-Like receptor, lipid peroxidation and apoptosis pathways. Identified biomarkers were further validated using Western blot and qPCR.

RESULTS : Heterogeneous lipid A structures of *P. gingivalis* showed strikingly different immuno-inflammatory activity. $PgLPS_{1690}$ induced pro-inflammatory cytokines IL-6, IL-8, TNF-α, GM-CSF. In contrast, $PgLPS_{1435/1449}$ induced anti-inflammatory molecules (SOCS box) and reduced lipid peroxidation pathway molecules such as perilipin and superoxide dismutase. $PgLPS_{1690}$ activated apoptosis (Bax-Caspase) related pathway whereas $PgLPS_{1435/1449}$ inhibited. Interestingly, we discovered that PgLPS isoforms utilize different mechanisms (TLRs, signal transduction pathways) for the modulation of host innate immune system which could account for aforementioned observation.

CONCLUSION : We have for the first time unraveled the molecular mechanism behind heterogeneous PgLPS isoforms which explain the ability of *P. gingivalis* to disguise host immune response to survive and proliferate in gingival tissues as a periodontal pathogen. Novel pathogenic mechanism described herein will certainly lay a new foundation to develop novel strategies for periodontal diseases (HKU766909M to LJJ).

06-1

Characteristics of toothbrushing in 4-5 year-old children in Selangor Malaysia

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BACKGROUND, RATIONALE AND STUDY OBJECTIVES : This study aims to characterize toothbrushing habits in 4-5 year-old children in Selangor Malaysia and determine the risk of ingestions, if any, associated with the toothbrushing characteristics.

DESIGN AND EXPERIMENTAL METHODS USED : Three hundred and fifty 4-5 year-old subjects were recruited for a questionnaire survey and subsequently 200 were subsampled for biochemical determination of toothpaste ingestion. The QA administered, to parents, enquired on history and characteristics of toothbrushing habits. Toothpaste ingestions were determined by the method of difference between toothpaste dispensed and residual toothpaste on the brush, taking into account the expectorated amount.

ESSENTIAL RESULTS : Three hundred and fifteen 4-5 year-old siblings responded to the QA survey, 174 returned specimens for determination of toothpaste ingestions and 159 respondents furnished complete QA and specimens findings.

Mean age of starting to brush teeth, brushing on their own and starting to use toothpaste was 31.5±15.0 months, 44.6±14.1 months, 35.3±15.5 months respectively. Most subjects brushed once (45.3%) or twice a day (41.1%). About half (48.4%) of subjects put approximately half length, a quarter (23.9%) put less than 1/4 length of the head of toothbrush with toothpaste. Most subjects claimed not to swallow toothpaste (90.1%) or the rinses (86.6%) although substantial proportion mentioned liking the taste of toothpaste (60.4%). Some subjects claimed they have been told they can swallow toothpaste (16.8) while most have been specifically told they cannot (80.7%). Most subjects claimed they were supervised during toothbrushing (82.3%), most commonly by their mothers (78.0%), but the supervision received was variable.

The mean amount of fluoride ingested from toothpaste was 226.9ug per day. This was correlated to amount of toothpaste dispensed ($r=0.689$. $p<0.001$), frequency of brushing ($r=0.282$, $p<0.001$), type of toothpaste used (Fluoridated vs non-Fluoridated) ($r=0.188$,$p=0.019$) and amount of fluoride in rinsings ($r=0.259$, $p<0.001$).

CONCLUSION : There is thus need to emphasise the importance of limiting the amount of toothpaste dispensed to reduce risk of excessive toothpaste ingestions.

06-2

Stem cells from human exfoliated deciduous teeth (SHED): trend analysis

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BACKGROUND AND RATIONALE : Stem cells from human exfoliated deciduous teeth (SHED) is a population of mesenchymal stem cells (MSC) and has been of interest in recent years due to the ease of obtaining deciduous teeth. These cells show STRO-1+ surface marker expression. However, no study has been done to investigate the extent of the ability for SHED to retain its stem cell characteristics.

OBJECTIVES : To isolate SHED and identify the cell cycle of STRO-1+ cells and the STRO-1+ expression across culture passages.

DESIGN AND EXPERIMENTAL METHODS : Deciduous teeth (n=56) were procured from children after dental treatment. Using mechanical and enzymatic disaggregation, cell suspensions of MSCs were isolated from dental pulp tissue and expanded in tissue culture flasks. Propidium Iodide was used to analyse the cell cycle of STRO-1+ cells of successfully isolated cell lines (n=8). Pearson Correlation was used to identify the pattern of STRO-1+ expression from passage P3 to P10 for each cell line.

ESSENTIAL RESULTS : Most of the STRO-1+ cells (59.40%) were able to proliferate and two cell lines showed high STRO-1+ expression (>90%) up to the 10^{th} culture passage.

CONCLUSION : Viable MSCs with proliferative capacity were isolated from exfoliated deciduous teeth. High STRO-1+ expression in subsequent passages showed that the isolated MSCs retained its stem cell characteristics.

07-1

The effect of recasting on the quality of pure titanium castings by MgO-based investment

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BACKGROUND: Titanium (Ti) has many advantages, such as excellent biocompatibility, high corrosion resistance, and good mechanical properties. Because of these advantages, Ti has been used extensively in dentistry for recent years. However, molten Ti is highly reactive with traditionally dental mold materials at elevated temperatures. Recently specially formulated mold materials containing MgO, Al_2O_3, ZrO_2, Y_2O_3, as refractory, were developed for titanium casting. Among these materials, MgO-based investment is easy to manipulate and can produce high quality of Ti casting.

STUDY OBJECTIVE: In the dental laboratory, surplus alloy is commonly reused from the initial casting for economic reasons and to avoid the exploitation of natural resources. Nevertheless, there was no study investigated the effect of the recasting pure Ti. The aims of this investigation were to evaluate the possibility of casting the reused pure titanium by using MgO-based investment material.

MATERIALS AND METHODS: The MgO-based investment (Selevest CB, Selec Co., Japan) and zirconia powder are prepared. Ti (CPT Dental titanium, Ohara Co.) ingot is casting by an automatic argon-casting machine (Castmatic-S, Iwatani Co., Japan). The castings of the first generation are used totally new Ti ingots. In the second generation, 50wt% new Ti combined with 50wt% surplus as the ingot. Marginal accuracy, average surface roughness, VHN, porosity and SEM are analyzed after casting.

RESULTS: Little small internal porosity was found in groups. The average marginal discrepancy and average surface roughness showed no significant difference between two groups ($p>0.05$). In VHN, there was no significantly difference at interface between Ti casting and mold material ($p>0.05$). From micro-chemical analyze, the reaction layer in the both group is no significantly difference.

CONCLUSION: Recasting of the pure titanium seems possible and workable when appropriate mold material is used.

08-1

Oral impacts on quality of life in adolescent cleft patients

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BACKGROUND : Maintaining an optimal oral health in cleft patients may be difficult due to the altered anatomy of the cleft area. No data is currently available on the prevalence of oral conditions and oral health-related quality of life among early-adolescents with cleft lip and/or palate in Thailand.

OBJECTIVES : The aims of this study were to determine the levels of dental caries, periodontal disease and oral health related quality of life in subjects with cleft lip and/or cleft palate as compared with non-cleft control subjects.

METHODS : A cross-sectional study was conducted among early adolescents aged 10-14 years. Subjects included 68 oral cleft patients attending the Center for Cleft Lip - Palate and Craniofacial Anomalies at Khon Kaen University, Khon Kaen, Thailand. One-hundred-and eighteen controls were randomly selected from two schools in surrounding areas. The Child-Oral Impact on Daily Performance (C-OIDP) index was administered prior to an oral examination.

RESULTS : The decayed, missing and filled teeth (DMFT) index in the permanent teeth, plaque index (PI), and gingival index (GI) scores were significantly higher in the children with cleft than in the controls. The C-OIDP mean impact score between both groups were not significantly different, but the cleft children with impacts had a higher mean impact score (11.87) than that of the controls (8.63). The intensity of impact at moderate to very severe level in most (81.0%) of the cleft children was higher than in controls (56.9%). The impact score in the cleft children was high for speaking (4.50) and emotion control (4.25). The main causes of those impacts included having oronasal fistula, sensitive tooth, malposed tooth and deformity of mouth or face.

CONCLUSION : The cleft children had higher level of dental caries and poorer oral hygiene than the controls. They also had higher intensity of impact and impact score than the controls.

08-2

Oral impacts affecting daily performance in Thai secondary school students

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BACKGROUND : Clinical measures reflect only one aspect of oral health status. Several socio-dental indicators have been used to assess the oral impacts on several aspects related to quality of life. However, there has been limited information regarding the oral impacts on quality of life in adolescents of Thailand.

OBJECTIVE : This study aimed to assess the prevalence of oral related impacts on the quality of daily life in Thai secondary school students.

METHODS : A cross-sectional study was conducted in Kaennakorn Wittayalai School, Khon Kaen, Thailand. Two hundreds and sixty eight students aged 15 years were selected to participate in this study. The impacts of oral health were assessed by using The Oral Impacts on Daily Performances (OIDP). It consists of 8 items that assess the impact of oral conditions on basic activities and behaviors that cover the physical, psychological, and social dimensions of daily living. Students were clinically examined and a self-administered questionnaire was used to identify health behaviors, oral care and dental attendance.

RESULTS : Forty percent of students had at least one OIDP oral impact. Thirty percent of those with impacts had 1-3 daily performances affected (out of 8 performances) The most common performance affected was eating (15%), tooth cleaning (12%) and emotional stability (11%). Major role activity, contact with people and speaking performances had high frequency of impacts. Sleeping, smiling and major role activities were rated as high severity. Pain, discomfort and dissatisfaction with appearance were mainly symptoms that caused almost every impact. Tooth decay, toothache and tooth position were the major causal oral condition of all aspects of performance.

CONCLUSION : Oral impacts that affected quality of life of Thai secondary students were relatively common but not severe. Most students in this study were concerned about esthetic.

09-1

Genome wide screening of mesenchymal signalling molecules involved in epithelial differentiation during palatogenesis

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In mice palatogenesis, along with the palatal fusion, dorsal and ventral epithelia of palatal shelves are differentiated respectively. Based on the key concept of epithelial-mesenchymal interactions during epithelial morphogenesis, palatal epithelium would be regulated by palatal mesenchymal factors. At E14, prior to palatal fusion, dorsal and ventral region specific palatal mesenchymal tissues were collected using Laser Micro-Dissection. After the collection of palatal tissue using LMD, total RNAs were harvested and analyzed by the genome wide screening method. Over 2-fold alterations genes were selected and examined using RT-qPCR and in situ hybridizations. Tissue specific expression patterns of mesenchymal factors suggest that they would play important roles in epithelial differentiation with the region specific manner. These expression patterns of mesenchymal factors would be a plausible answer for understanding the pathogenesis of cleft palate.

09-2

Signaling modulations of Rgs19 in palatal EMT process

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Palatal development is one of the crucial events in craniofacial morphogenesis according to the significant signaling pathway including the out growth, elevation and fusion of the palatal shelves. In the fusion of palatal shelves, an epithelial to mesenchymal transition (EMT) is a fundamental process to achieving the proper morphogenesis of the palate. The mechanisms of the EMT have been reported to be the processes of migration, apoptosis or general EMT through modulations through various signalling molecules. Rgs19, which is a regulator of the G protein signaling (RGS) family through GTPase activity, showed interesting epithelial expression patterns in various organogeneses including palatal development. To examine the precise developmental function of Rgs19 in palatogenesis, this study employed a loss of function study using AS-ODN treatments during *in vitro* palate organ cultivations. Three dimensional reconstructions with the immunostaining of pan-Cytokeratins showed a significant decrease in the number of apoptotic cells in medial edge epithelium (MEE) after knocking down Rgs19. These retarded patterns of palatal fusion would be result from the altered expression patterns of the candidate genes including Axin2, CyclinD1, Lef1, Twist, Snail, Slug and TGFb3. Overall, Rgs19 modulates the palatal EMT process through the apoptotic pathway and signaling interactions between the Wnt responsive and EMT related genes.

10-1

The site of the lesion should be considered when identifying biomarkers for OSCC

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The ability to provide effective diagnosis, prognosis and targeted therapy remains limited in oral squamous cell carcinoma (OSCC) due to lack of understanding of the molecular pathogenesis underlying oral cancer development. Notably, clinical evidence suggests biological behaviour, survival and response to treatment in patients with OSCC from different anatomical locations are distinct, which could in part be caused by the activation or/and inactivation of different pathways. To further confirm this hypothesis, we studied gene expression of OSCC from different sites of the oral cavity to determine if there were significant differences in the pattern of gene expression. 63 OSCC and 35 site-matched non-malignant formalin-fixed paraffin-embedded tissues were used in microarray experiments (DASL, Illumina) to identify gene expression patterns of OSCC from the buccal, gum and tongue. Using GeneSpring, differentially expressed genes were identified and validated through quantitative polymerase chain reaction (qPCR). Oral cancer cell lines were used to study the function of differentially expressed genes. Using Principal Component Analysis, we demonstrated that OSCCs of the buccal and the gum segregate distinctively from one another while the gene expression of OSCC from the tongue was heterogeneous. Differentially expressed genes which are commonly altered in all sites and those that were distinct to a particular site were ascertained. Hierarchical Clustering analysis and Pathway Enrichment analysis using DAVID indicated that there were site specific gene expression signatures. Focusing on a gene found to be enriched in OSCCs of the tongue, we demonstrated that the overexpression of this gene promoted migration and invasion of OSCC cells. This study suggests that genetic progression of OSCC in the different sites are distinct, thus cautioning the generalization of OSCC when identifying biomarkers for diagnosis, prognosis and therapy. Furthermore, specific genes may confer different cancer traits that may explain the clinical differences seen in different sites of OSCC.

10-2

Synthesis and characterization of nanosilica as filler for the novel dental resin system

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The aim of this work is to synthesis nanosilica via sol-gel method for the use as fillers in urethane acrylate macromer (UMA), a novel resin system based on palm oil polyol. The monodispersed spherical nanosilica were synthesized using tetraethoxysilicate (TEOS) as precursor and ammonium hydroxide as catalyst. Then the nanosilica were silanized with gamma-methacryloxypropyltrimethoxysilane (MPS) to reduce agglomeration of nanosilica. The structural properties, surface properties and the chemical structure were investigated by Fourier Transform Infrared Spectrometer (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), Nuclear Magnetic Resonance (NMR) and BET surface area analysis.The size of particle was measured using zeta sizer and also SEM. XRD powder of nanosilica confirms the amorphous nature of the substance. BET analysis measured the total surface area and the pore size of the resulting material. FTIR and NMR spectrums shows the presence of nano silica. The silanaized nanosilica was blended into the UAM resin system and the surface morphology, roughness and hardness were evaluated.

11-1

Retained microbial flora in toothbrushes following usage of herbal & fluoridated toothpastes

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BACKGROUND : Effective plaque removal is the cornerstone for good oral hygiene as it plays a significant role in preventing periodontal disease and caries. The toothbrush is a valuable tool for effective plaque removal. Retention and survival of oral microorganisms on toothbrushes represent a possible source of contamination. The usage of herbal toothpaste is increasing with anecdotal evidence suggesting their benefits.

HYPOTHESIS : Herbal toothopastes have the same effect as fluoridated toothpastes on the amount of retained microbial flora in toothbrushes.

DESIGN AND EXPERIMENTAL METHODS : Thirty users of commercially available herbal and fluoridated toothpastes and having a plaque score less than 30% were recruited. They were randomly divided into 2-groups of 15 each. Group-A was provided with a commercially available herbal toothpaste and Group-B a commercially available fluoridated toothpaste. Each subject was given a toothbrush with the same characteristics. A standard brushing technique was recomended and supervised over 1-week. Once collected, the each brush was dipped in sterile Phosphate-Buffered-Saline (PBS) and votexed for 3-mins and the suspension was centrifuged for 10-mins (1500rpm). The pellate was re-suspended in 1ml PBS and serial dilutions were made using PBS. An inoculum of 25μl from the 10^4 dilution was plated in triplicate in blood-agar. The above procedure was individually performed for all brushes. Cultures were incubated aerobically at 37°C over 48h. The total bacterial colonies were counted. The above was repeated with the toothpaste alternated between the two groups.

RESULTS : The mean total bacterial count of fluoridated toothpaste users was 17.68±3.66 while the count for herbal toothpaste users was 12.35±2.09. A significant difference between the total bacterial counts of fluoridated and herbal groups was observed ($p<0.01$).

CONCLUSION : Herbal toothpaste seems effective in reducing retained flora in tooth brushes. Further investigations are necessary to deterimine the effect of herbal toothpaste on plaque control.

12-1

Effect of Ca/P concentration on the surface topography and micro-mechanical properties of Ti-Alloy micro-arc oxidation coating

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BACKGROUND : The relationship between micro-mechanical properties of the coating of titanium alloy produced by micro-arc oxidation (MAO) and the electrolyte formula has not been well investigated.

OBJECTIVE : To explore the effect of Ca and P concentration in the electrolyte on the micro-mechanical properties of the titanium alloy coating formed by MAO technique.

METHODS : 20 disc-shaped Ti-6Al-4V specimens of 10mm diameter and 1mm thickness were equally divided into 4 groups. The surfaces were treated by MAO technique in electrolytic solution containing calcium acetate (CA) and β-glycerol phosphate disodium salt pentahydrate (β-GP). The concentration of β-GP was fixed at 0.02 mol L^{-1}, the concentrations of CA were set at 0.05, 0.1, 0.2 and 0.3mol L^{-1} respectively for the 4 testing groups. The morphology of the MAO coatings were observed by scanning electron microscopy (SEM) and the composition was detected by an energy dispersive spectroscope (EDS) incorporated in the SEM. The phase and the microstructure of the coating were analyzed by XRD. The micro hardness and elastic modulus were measured by atomic force microscope (AFM).

RESULTS : Under SEM, the structure of the MAO coating was observed to become more even and the crystal size became smaller with the decrease of Ca^{2+}. XRD pattern showed that the oxide coating was mainly rutile and hydroxyapatite. EDX and nanoindenation results showed that with the increase of Ca^{2+} in the electrolyte, the concentration of Ca in the coating increased accordingly, while the concentration of P decreased obviously, Ca/P ratio increased from 0.52 to 2.44 consequently, while the elastic modulus of the MAO coating decreased significantly, from 60.5 to 32.0GPa, and the hardness decreased from 3.56 to 0.72GPa.

CONCLUSIONS : Ca and P concentration in the electrolyte have an effect on the surface tomography and micro-mechanical properties of the MAO coating.

12-2

Anti-inflammatory effect of erythropoietin on human dental pulp cells

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BACKGROUND : Erythropoietin (EPO) plays a critical role in controlling immune and inflammatory responses. However, its effect on pulpal inflammation has not been clarified.

OBJECTIVE : This study aims to evaluate the effects of EPO on the proliferation, apoptosis as well as the expressions of inflammatory cytokines in human dental pulp cells (DPCs) stimulated with lipopolysaccharide (LPS).

METHODS : The proliferation and hydrogen peroxide (H_2O_2)-induced apoptosis of DPCs were measured using CCK-8 assay and flow cytometry, respectively. The protein expressions of EPO and EPO-R in DPCs by LPS stimulation were detected by Western blot. Moreover, the production of inflammatory cytokines interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) in LPS-stimulated DPCs were determined using Enzyme-linked immunosorbent assay.

RESULTS : EPO increased the proliferation of DPCs on days 1 and 2 ($p<0.05$), and 20U/mL had the greatest effect on the proliferation of the cells. EPO significantly reduced the apoptosis of H_2O_2-induced DPCs from 72.6%±0.05% to 25.2±0.15% ($p<0.05$). The expressions of EPO and EPO-R were upregulated in DPCs by LPS stimulation, reached the peak at the 6 hours ($p<0.05$). Furthermore, EPO decreased the secretion of IL-1β and IL-6 in LPS-stimulated DPCs ($p<0.05$), but had no effect on the production of TNF-α ($p>0.05$).

CONCLUSIONS : EPO enhanced the proliferation of DPCs and reduced the apoptosis of H_2O_2-induced DPCs. LPS increased EPO and EPO-R protein expressions in DPCs. EPO inhibited LPS-stimulated inflammatory cytokines' expressions in DPCs. These findings indicated the potential anti-inflammatory activity of EPO in pulpitis.

13-1

Flavonoids promoting osteogenic differentiation of dental pulp stem cells

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Flavonoids are pigment of plants and are responsible for growth, development, and defense. The biological activities of these compounds include antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-cardiovascular disorder, etc. In our previous studies, we have successfully screened the effective flavonoids for inhibiting osteoclastogenesis and for increasing the mineralization process of osteoblast cells. Beside the osteoclast and osteoblast, the stem cells also play an important role in the process of bone formation. Stem cells have great potential in wound healing due to their self-renewal and multilineage differentiation potential. Increasing studies are addressed on stem cells from dental tissues since the successful isolation and culture of mesenchymal stem cells from dental tissues. Therefore, the current study is engaged in screening the effective flavonoids for the osteogenesis of dental pulp stem cell (DPSC), and to investigate the effect of these drugs on cell physiology, intracellular signal transduction, and the regulation of osteogenesis-related proteins. The analysis of alkaline phosphatase (ALP) activity is applied in screening for effective flavonoids. Among 19 flavonoids, only two flavonoids, termed Flav-1 and Flav-4, could increase ALP expression of DPSC. It is interesting that the isomers Flav-2 and Flav-15 failed to exhibit the same effect, although these four flavonoids (Flav-1, Flav-2, Flav-4, Flav-15) are only different in the amount or the position of OH groups. In addition to ALP expression, Flav-1 and Flav-4 are also confirmed to increase the activation of osteogenesis-related proteins such as Runx2 and collagen I. These results demonstrate the flavonoids Flav-1 and Flav-4 are potential therapeutic drug candidates for bone regeneration. This study is beneficial in drug development for DPSC-based tissue regeneration.

14-1

A biomimetic strategy to induce remineralization of partially demineralized dentine

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OBJECTIVE : The aim of this study is to induce remineralization of partially demineralized dentine by introducing phosphate groups onto the collagen matrix of dentine through sodium trimetaphosphate (STMP) treatment. This strategy is based on the hypothesis that phosphate groups introduced onto collagen can mimic a nucleating role of the phosphorylated noncollagenous proteins binding to collagen *in vivo*.

METHOD : The partially demineralized dentine sections were randomly divided into three groups (n=6) according to different treatments. The samples were firstly phosphorylated by STMP treatment and then treated with saturated calcium hydroxide, which were subsequently exposed to remineralizing solution. The morphology, chemical components and surface properties of the samples were investigated by Fourier-transform infrared (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), contact angle and zeta potential measurements.

RESULTS : The ATR-FTIR, XRD and SEM results indicated that the STMP-treatment alone did not induce significant remineralization of demineralized dentine, while saturated $Ca(OH)_2$-treatment followed by STMP-treatment resulted in significant remineralization. The surface free energy component, Lewis base (γ^-) of the samples decreased after demineralization and then increased with phosphorylation. In contrast, the interfacial free energy (γ_{SL}) of the hydrated samples increased after demineralization and decreased conspicuously with phosphorylation treatment.

CONCLUSION : Phosphorylating collagen matrix in demineralized dentine aids in obtaining favorable surface characteristics (i.e. highly negative charge and low interfacial free energy between substrate and medium) for mineral crystal nucleation, which when combined with $Ca(OH)_2$ pretreatment, is a promising method to remineralize superficially demineralized dentine lesions.

15-1

Tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*

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The anticariogenic properties of tea polyphenols have been documented in humans and experimental animals. Tea polyphenols, especially epigallocatechin gallate (EGCg), have been shown to inhibit *in vitro* growth and glucosyltransferases (GTFs) activity of *Streptococcus. mutans*. However, their effects on biofilm and cariogenic virulence factors of *S. mutans* other than GTFs have not been well documented. We hypothesize that EGCg reduces acid production and acid tolerance of *S. mutans* by suppressing these virulence factors at the transcriptional and enzymatic levels.

OBJECTIVES : To investigate the biological effect of EGCg on acid production and acid tolerance of *S. mutans*.

METHODS : The antimicrobial effect of EGCg against *S. mutans* UA159 planktonic and biofilm cultures was determined by micro-dilution method in 96-well microtiter plate. Acid production and acid tolerance of *S. mutans* UA159 grown in the presence of sub-MIC levels of EGCg (1.95-15.6μg/ml) were determined *in vitro*. The effects of EGCg on acidurity and acidogenicity properties of *S. mutans* were also examined at transcriptional and enzymatic levels.

RESULTS : EGCg inhibited growth of *S. mutans* planktonic cells (MIC, 31.25μg/ml; MBC, 62.5μg/ml), and the formation and viability of biofilm ($MIBC_{90}$, 15.6μg/ml; $SMIC_{80}$, 625μg/ml). At sub-MIC levels, EGCg inhibited acidogenicity and acidurity of *S. mutans* cells. Analysis of data obtained from real-time PCR showed that EGCg significantly suppressed the *ldh*, *eno*, *atpD*, and *aguD* genes of *S. mutans* UA159 associated with acidurity and acidogenicity ($p<0.05$). Inhibition of enzymatic activity of F_1F_0-ATPase and lactate dehydrogenase was also noted (IC_{50} between 15.6μg/ml and 31.25μg/ml).

CONCLUSION : In addition to its antimicrobial activity, EGCg may represent a natural anticariogenic agent by suppressing specific cariogenic virulence factors of *S. mutans*.

16-1

Evaluation of quality of life according to temporomandibular disorder associated symptoms in dental hospital worker

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BACKGROUND AND RATIONALE : Temporomandibular disorder (TMD) is relatively prevalent disease and quality of life may be impaired in TMD patients. Like general population, dental hospital workers are also exposed to the risk of TMD. But, many of them tend to overlook or tolerate their symptoms for lack of time and interest. Therefore, problems may become more serious, causing interference of performing task and decrease of quality of life.

STUDY OBJECTIVE : The aim of this study was to obtain data for TMD prevalence in dental hospital workers and to evaluate quality of life according to TMD associated symptoms.

METHOD : Subjects were recruited from Wonkwang University Dental Hospital. After consent, subjects completed quality of life questionnaire (Korean version of WHOQoL; WHOQoL-K) and were evaluated for subjective and objective signs and symptoms of TMD in accord with the Research Diagnostic Criteria (RDC/TMD). Subjects were classified into 4 groups. (1) normal group (2) intracapsular pain (ICP) group, (3) masticatory muscle pain (MMP) group, and (4) wide spreading pain group.

RESULT : The result of the study indicated that TMD associated symptoms negatively influence the quality of life in dental hospital worker.

CONCLUSION : TMD-associated symptoms can deteriorate quality of life in dental hospital worker. Future effort to make protocol for proper management is needed.

17-1

Regulation of both osteoblastic and myogenic differentiation by proteasome inhibitor bortezomib

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In eukaryotic cells, degradation of most intracellular proteins is carried out by the ubiquitin-proteasome pathway. Recent observations suggest that bone metabolism is regulated by the ubiquitin-proteasome pathway, and certain proteasome inhibitor has been reported to induce differentiation of osteoblasts in vitro and in vivo. The clinical efficacy of bortezomib, a 26S proteasome inhibitor used as an anticancer drug in the treatment of multiple myeloma, has been linked to an increase in bone markers. However, until now, little was known that the molecular basis for action of proteasome inhibitor on the regulation of osteoblastic differentiation. In the present study, we show that bortezomib, but not lactacystin, epoxomicin, or MG-132, not only induces osteoblastic differentiation but also inhibits myogenic differentiation in C2C12 cells in culture. Bortezomib also inhibited myogenic gene expression such as myogenin and muscle creatine kinase, and induced osteocalcin and alkaline phosphatase gene expression. To investigate the mechanisms by which bortezomib activates osteocalcin transcription, transient transfection of osteocalcin (OG2) promoter-luciferase constructs with bortezomib treatment resulted in a significant increase in luciferase activity. Mutation of not OSE1 but OSE2 diminished bortezomib-induced this activity, implying that the regulation of transcriptional activity through interaction with the Runx2 via OSE2 in OG2 promoter by bortezomib. However, bortezomib did not induce their transcription activities using reporter construct that have BMP responsive elements of Id-1 gene, suggesting that bortezomib does not modulate Smad1/4-dependent BMP signaling pathway. Also, β-catenin is a well-known target of the ubiquitin-proteasome pathway. However, bortezomib did not alter transcriptional activities of β-catenin dependent Top-flash reporter and canonical Wnt signaling target gene expression. These observations indicate that the function of the proteasome in controlling degradation of poly-ubiquitinated signaling molecules such as Runx2 plays an important role in osteoblast differentiation.

18-1

Functional role of Platelet-derived growth factors involved in ameloblasts and odontoblasts proliferation

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During the process of tooth development, cell proliferation is well regulated to form functional shape and size of tooth germ. Platelet-derived growth factors (PDGFs) play crucial roles in organogenesis including tooth. Previously, we have reported that PDGFs play impotant roles for tooth morphogenesis. Here, we focused on the roles of PDGFs on the proliferation of ameloblasts and odonotoblasts. RT-PCR and immunostaining demonstrated that PDGF ligands, *PDGF-A and PDGF-B, and* their receptors, *PDGFRα, and PDGFRβ,* were expressed during the initial stages of tooth development. Addition of AG17, a tyrosine kinase inhibitor specific for PDGF receptor signaling, suppressed incorporation of bromodeoxyuridine (BrdU) in both dental epithelial and mesenchymeal cells in tooth germ organ culture. As the results, growth and cusp formation of molar tooth germ were inhibited. Next, we tested whether PDGF-AA or -BB affect dental epithelial cell differentiation. Dental epithelial cell line, SF2 cells were cultured with PDGF-AA or -BB. PDGF-AA, but not -BB, was strongly induced the expression of ameloblastin gene. Further, we examined the effects of PDGF-AA and -BB on cell proliferation of the dental pulp stem cell line SP cells. PDGF-BB promoted SP cells proliferation, whereas PDGF-AA did not. Further, we found that PDGF-BB induced the phospholylation of ERK1/2, but not of p38. In conclusion, our results indicate that PDGF-AA accelerates ameloblast differentation and PDGF-BB promotes odontoblast proliferation though ERK1/2 pathway, indicating that PDGFs have crucial roles in tooth development.

19-1

Pharmacological manipulation of oral mucosa keratinocytes by blocking ALDH activity

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Our ultimate goal is to produce a "robust" oral mucosa graft that will be used for reconstruction of major oral mucosa defects. Unmanipulated human oral keratinocytes are currently used to fabricate autologous oral mucosa grafts (*ex vivo* produced oral mucosa equivalents: EVPOME). We previously reported that aldehyde dehydrogenase (ALDH) is a negative marker of oral mucosa progenitor/stem cells because ALDH expression lacked in basal layer but was present in lower suprabasal layer in native oral mucosa. Since ALDH plays a major role in biosynthesis of retinoic acid, a regulator of cellular proliferation, differentiation and survival, we hypothesized that blocking ALDH activity could prevent undifferentiated oral keratinocytes from committed terminal differentiation. The objective of this study was to maintain the undifferentiated state of oral keratinocytes by pharmacological manipulation. Primary oral mucosa keratinocytes were serially cultured in a chemically defined, serum-free culture medium (EpiLife®). Cells, in a monolayer culture, were treated with 100μM diethylaminobenzaldehyde (DEAB), an ALDH inhibitor, or 10nM all-trans retinoic acid (ATRA) for ALDH trans-activation for three days. The expression of α6 integrin and CD71 was analyzed by fluorescent activated cell sorter (FACS). Regenerative capability of oral mucosa (EVPOME) using an organotypic culture was also examined. ATRA treated cells showed a remarkable decrease of α6 integrin expression, resulting in losing the $\alpha6^{bri}$/$CD71^{dim}$ cell population, undifferentiated cells, as well as increment of $\alpha6^{dim}$/$CD71^{dim}$ cell population, differentiating keratinocytes. Histologically, ATRA treated cells developed a thinner, less-organized epithelial layer compared with untreated cells, consistent with the FACS analysis. In contrast, DEAB treated EVPOME formed a well-structured epithelial layer. In addition, immunohistochemical examinations showed a lesser expression of keratinocyte differentiation markers in the DEAB treated EVPOME. In summary, blocking ALDH activity by DEAB rendered oral keratinocytes less differentiated, implying this pharmacological manipulation might facilitate our ultimate goal.

19-2

Zoledronic acid induces S-phase arrest via a DNA damage response in normal human oral keratinocytes

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BACKGROUND : Bisphosphonates (BPs) provide an effective treatment for various bone diseases. However, Osteonecrosis of the jaws (ONJ) has drawn attention as one severe side effect for patients receiving BPs.

OBJECTIVE : Although BRONJ appears to originate in the bone, we hypothesized that changes in oral mucosa might also contribute to its pathogenesis. This study aimed to clarify the effects of zoledronic acid (ZOL) on human primary oral mucosa keratinocytes growing in a monolayer culture and a tissue-engineered construct.

DESIGN : We measured changes in the viability and proliferation of oral keratinocytes incubated with ZOL. Following the treatment with 10μM ZOL, the histological examination and immunohistochemical analysis for Ki-67, geminin, and phospho-histone (γ)-H2A.X were implemented in the tissue-engineered oral mucosa. This study also analyzed cell cycle distribution and apoptotic reaction by flow cytometry; the expression and alteration in cell cycle regulatory proteins and phospho-Chk1 and -Chk2.

RESULTS : ZOL suppressed cell viability and proliferation in a dose dependent manner. The tissue-engineered oral mucosa cultured with ZOL showed a thinner epithelium in which basal cells appeared less-organized, consistent with a significant reduction of the Ki-67-labeling index. In contrast, the geminin-labeling index rose significantly higher than that of the untreated one. In spite of a few apoptotic cells being found, ZOL induced S-phase arrest that was confirmed by alterations in the expression levels of cyclin A, B1, $p27^{KIP1}$, Rb and phospho-Rb. When MG132 was added to the ZOL treated cells, the expressions of these proteins were partially restored. Phospho-Chk1 expression and a significant increase in the labeling index of γ-H2A.X were also detected.

CONCLUSIONS : The present results indicated that 10μM ZOL treatment induces a DNA damage response in oral keratinocytes that activates the ubiquitin-mediated proteolysis of cell cycle regulators and arrests the cell cycle, resulting in repressive effects on cell viability, proliferation, and epithelial turnover.

19-3

Histological and immunohistological analyses of oral mucosal epithelial organization and kinetics compromised by Bisphosphonates

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Recently, great attention has been paid to Bisphosphonates (BPs)-related osteonecrosis of the jaw (BRONJ), as a severe side effect of BP therapy, which is characterized by exposed nonviable jaw bones. Although the pathogenesis of BRONJ remains unknown, BPs may have cytotoxic effects on not only the jaw bones but also an oral mucosa soft tissue. The main objectives in this study were to analyze the effects of both Zoledronic acid (ZOL) and Pamidronate (PAM) on oral mucosal epithelial organization and kinetics using an organotypic model. Primary oral mucosa keratinocytes and fibroblasts were serially cultured in a serum-free EpiLife® culture medium and 10% serum containing DMEM, respectively. A 3D oral mucosa substitute was prepared using passage number 3-5 cells. After contraction of a type I collagen gel repopulated with oral fibroblasts was completed, oral keratinocytes were seeded onto the top of the gel and cultured for seven days in a submerged condition. Subsequently, the substitute was raised to an air-liquid interface (A/LI) and cultured for another seven days to form an epithelial stratification. To test an epithelial organization of oral mucosa, the substitute was treated with 10μM of BPs during the period of A/LI. To examine the epithelial kinetics (*in vitro* wound healing: re-epithelialization), the oral mucosa substitute was treated with BPs for 7 days after the stratified epithelial layer was wounded. ZOL severely compromised the epithelial organization, resulting in a thinner, poorly-organized oral mucosa compared with untreated model. This attenuation was supported by a significant decrease of Ki-67 immuno-positive cells. The re-epithelialization was also impaired by BPs treatment. In contrast, the inhibitory effect of PAM was less severe than that of ZOL. In conclusion, BPs deteriorated the oral mucosal epithelial organization and impaired the re-epithelialization, suggesting the compromised oral mucosa tissue may be also associated with the onset of BRONJ.

19-4

Effects of mechanical loading on the condyle in a rat mandibular distraction model

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BACKGROUND : Progressive condylar resorption (PCR), an irreversible complication, is a factor for a late skeletal relapse after mandibular advancement for patients with jaw deformity. However, the etiology of PCR remains unclear.

OBJECTIVE : PCR has been hypothesized to occur at excess mechanical loading over the adaptive capacity of the condyle. Macro- and microscopical examinations were employed to demonstrate changes in the condyle in a rat overloading model with mandibular distraction.

EXPERIMENTAL DESIGN : A distraction device was employed to achieve unilateral mandibular distraction in 10-week-old Wistar rats, with the distraction at 0.2mm/12 h for 10 days after a 5-day latency period. The animals were perfused with 4% paraformaldehyde 1 day or week after distraction. The samples were scanned by μCT to reconstruct 3-dimensional images. Serial paraffin sections including decalcified tissue specimens were sagittally cut with a microtome. They were stained with hematoxylin and eosin (HE), and processed for histochemistry for and tartrate-resistant acid phosphatase activity (TRAP).

RESULTS : Micro CT observation showed erosive bone resorption in the anterior area of the condyle on the lengthened side, in particular 1 week after distraction. The lengthen side demonstrated thickening of the anterior band of the articular disk, thinning and disarrangement of chondrocytes in condylar cartilage, reduction of tissue metachromasia in the hypertrophic cartilage layer and expansion of the marrow cavity below the condylar cartilage. The condylar cartilage contained a larger number of intense TRAP reactive osteoclasts at the lengthened side. However, histologic and hitochemical features did not differ between the non-lengthened side and the control group.

CONCLUSION : Mechanical loading by mandible distraction caused various histological changes, in particular bone resorption in the anterior condyle area, indicating that overloading to the condyle is a key factor for PCR.

20-1

A crucial role of osteocytes in osteoclastic bone resorption during orthodontic tooth movement

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Several lines of evidence demonstrate the important role of osteocytes in bone remodeling induced by mechanical loading. Orthodontic tooth movement is a good model to understand bone remodeling induced by mechanical loading, because active bone resorption occurs at compression site followed by bone formation at tension site during tooth movement. To investigate the roles of osteocytes in bone remodeling induced by mechanical loading, we apply osteocyte-ablating mice, which express diphtheria-toxin (DT) receptor specifically in osteocytes by using DMP-1 promoter, and osteocytes are killed by injection with DT. Tooth movement was conducted by using orthodontic appliance. After tooth movement of upper first molar to mesial side, we compared the distance of tooth movement and the histological changes between mice with or without intraperitoneal DT injection (50μg/kg) on days 4, 8 and 12. To assess the distance of tooth movement, we took dental impression of upper jaws. The distance of tooth movement was expressed by the length of space between first and second molars measured on the dental impression. Histological analysis was performed using decalcified paraffin sections. DT injection increased the number of empty lacunae, which indicate osteocyte death, in alveolar bones. The distances of tooth movement in DT-injected mice tended to be smaller than that in DT-uninjected mice on days 4 and 8, but the distance in DT-injected mice became to be significantly smaller than that in DT-uninjected mice group on day 12. Interestingly, the number of TRAP-positive osteoclasts decreased in DT-injected mice after day 8, compared with that in DT-uninjected mice. These results suggest that osteocytes are involved in osteoclast formation in response to mechanical stress, and that osteocytes play a crucial role in mechanical load-induced bone remodeling during tooth movement.

21-1

Discovery of novel antifungal small-molecules in *Candida albicans*

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BACKGROUND : *Candida albicans* is a leading cause of nosocomial infections and the emergence of drug resistance *Candida* strains has become a major global concern. One method which prevents *Candida* from acquiring drug resistance is to develop new antifungal drugs.

OBJECTIVES : In the present study, we aimed to find out novel antifungal small-molecules by using small-molecule library screening method in *C. albicans*. Moreover we evaluate the effect of small-molecules detected by library screening against *Candida* biofilm.

EXPERIMENTAL METHODS : *C. albicans* SC5314 strain was used in this study. Initially, library screening of Lopac^{1280} (Sigma), which contains 1,280 pharmacologically active compounds, was performed using CLSI method. Antifungal effect of small-molecules was evaluated by using XTT reduction assay. After library screening, antifungal susceptibility test of *C. albicans* in planktonic, adhesion and biofilm modes was performed against small-molecules which were detected by library screening.

RESULTS : The library screening by CLSI method detected 35 antifungal drug candidates. 26 candidates had fungistatic effect and 9 candidates had fungicidal effect against *C. albicans*. Especially, 5 compounds had strong fungicidal effect, therefore antifungal susceptibility test was performed against that 5 compounds. All compounds had fungicidal effect against planctonic, adhesion and biofilm mode of *C. albicans*.

CONCLUSIONS : These results suggest that the cell-based screening by the CLSI method could identify candidates for antifungal compounds and make it a promising tool for finding novel synthetic antifungal drugs. Moreover, detected compounds by this method have fungicidal effect against candidal biofilm and might become a novel antifungal drug.

22-1

Novel effects of CCN3 that may direct the differentiation of chondrocytes

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BACKGROUND : Chondrocytes fated to form articular and transient cartilages seem to belong to distinct cohorts of progenitor cells. Nevertheless, the local molecules responsible for affecting the chondrocyte phenotype are still a field of intense debate. The cells in femoral distal epiphyses of five days old rats show uniform aggrecan and tenascin-C localizations, which indicates no distinct subpopulations that yield to ossification and articular cartilage, this finding brings a question; what are the effector molecules acting *in loco* telling some cells to enter endochondral ossification pathway and others to remain as cartilage? This study was conducted to provide an answer to this question.

HYPOTHESIS : In this context, CCN Family of regulator factors, a family that plays important roles in cartilage metabolism should be noted. Here, we posited that CCN3, a molecule whose effects on epiphyseal cartilage are not well characterized, might play a role in this process.

DESIGN : Localization of CCN3 in distal femoral epiphysis before secondary ossification was analyzed by immuhistochemistry and semi-quantified by immunoblotting. Effect of exogenous CCN3 on these epiphyseal cells was analyzed *in vitro* regarding changes in chondrocytic marker mRNA, proteoglycan synthesis and cell proliferation. To further confirm CCN3 effects, loss-of-function in a CCN3 downregulation scenario was evaluated by knocking CCN3 down with an shRNA in the prechondrocytic cell line ATDC5.

RESULTS : CCN3 is expressed throughout the epiphyseal head of rats before secondary ossification. Exogenous CCN3 increased the mRNA expression of tenascin-C and aggrecan, while repressing type X collagen mRNA expression in primary cells from epiphyseal heads. In parallel to the effects on matrix metabolism, exogenous CCN3 repressed cell proliferation. The knock-down experiment showed a loss-of-function regarding matrix metabolism in the ATDC5 cells.

CONCLUSION : Our results indicate a pivotal role of CCN3 during the process of chondrocytes differentiation towards articular or temporary cartilage.

23-1

Effect of advanced glycation end-products on calcified tissue formation in rat dental pulp cell cultures

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BACKGROUND AND OBJECTIVE : Amorphous calcification frequently appears in dental pulp tissues of diabetic patients, however, its pathogenesis is poorly understood. We previously found that pathologic pulp calcifications such as pulp stone and thickened predentin tend to occur in diabetic rats. It is conceivable that advanced glycation end-products (AGEs), final products of the Maillard reaction, are closely correlated with vascular calcification in diabetes. The aim of this study is to investigate whether or not AGEs can upregulate calcification in rat dental pulp cell cultures.

METHODS : Dental pulp cells from rat maxillary incisors were cultured as described by Kasugai *et al*. (*Arch Oral Biol* 1993). AGE-BSA were prepared by a modification method of Takeuchi *et al*. (*Mol Med* 1999). Firstly, RT-PCR was performed to determine mRNA expressions of AGE receptor (RAGE), osteopontin (OPN) and osteocalcin (OCN) using dental pulp tissues of diabetic and nondiabetic rats. Secondly, the effect of AGE-BSA (200 and 1000μg/ml) on alkaline phosphatase (ALPase) activity was determined using the cultures of rat dental pulp cells and gingival fibroblasts, and then the amount of calcium deposition was analysed by von Kossa staining. The effect of AGE-BSA on mRNA expressions of RAGE, OPN and OCN in the pulp cells were also determined by RT-PCR.

RESULTS : Expressions of RAGE, OPN and OCN mRNAs in dental pulp tissues were higher in diabetic than in nondiabetic rats. In cultured dental pulp cells, AGE-BSA significantly increased ALPase activity and calcium deposition. In cultured gingival fibroblasts, AGE-BSA did not change ALPase activity and calcium deposition. The increase in mRNA expressions of RAGE and OPN was also observed in the pulp cells.

CONCLUSION : AGE upregulated osteoblastic markers of dental pulp cells, suggesting that AGEs are involved in pathologic pulp calcification which are frequently observed in diabetic conditions.

24-1

Cot/Tpl2 is functionally involved in the stretch-induced down-regulation of PPARgamma1 expression in osteoblasts

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Mechanical stimuli are known to regulate osteoblast functions. Peroxisome proliferator-activated receptor gamma (PPARgamma) is a nuclear hormone receptor, playing important roles of cell proliferation, differentiation, and survival in a wide range of tissues and cell types. PPARgamma consists of two isoforms PPARgamma1 and PPARgamma2 by the result from the alternative splicing and promoter use. However, the functional roles of PPARgamma1 and PPRAgamma2 in osteoblasts and relation with mechanical stimuli are mostly unknown. Here, we show the down-regulation of PPARgamma1 expression in the osteoblasts in response to mechanical stimulus using a cyclic cell stretcher system. PPARgamma1 was detected by reverse transcription polymerase chain reaction in the primary osteoblasts prepared from newborn mouse calvarie, whereas the PPARgamma2 transcript was totally absent. The presence of PPARgamma1 was also confirmed in MC3T3-E1, a mouse osteoblast cell line. Mechanical stretch reduced the expression level of PPARgamma1 mRNA and protein in primary osteoblasts. We then examined the signaling pathway inducing the reduction of PPARgamma1 expression by mechanical stretch. The mitogen activated protein kinases (MAPKs) are known to be crucial molecules in mechano-transduction, whereas the whole picture of the mechano-signaling pathways remains to be elucidated. We explored the role of Cot/Tpl2, a MAPK kinase kinase (MAP3K), in the osteoblast response to mechanical stimulus. Stretch-induced extracellular signal-regulated kinase1/2 (ERK1/2) phosphorylation in the primary osteoblasts was inhibited by a specific Cot/Tpl2 kinase inhibitor (TKI). TKI treatment also inhibited the stretch-induced phosphorylation of ERK1/2 in MC3T3-E1. The primary osteoblasts from cot/tpl2$^{-/-}$ mice showed the attenuated phosphorylation level of ERK1/2 induced by the stretch. Furthermore, the stretch-induced reduction of PPARgamma expression level was attenuated by both TKI treatment and the gene-deficiency of *cot/tpl2*. These findings demonstrate that mechanical stretch induces the down-regulation of PPARgamma1 expression in osteoblasts, which is mediated by the activation of Cot/Tpl2-ERK pathway.

25-1

Newly developed carbonate apatite-chitosan scaffold for *in vitro* bone tissue engineering

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BACKGROUND : The natural biopolymer chitosan is currently regarded as a candidate for bone tissue engineering. We fabricated chitosan scaffold (ChS) by newly developed chitosan and decided suitable concentration of ChS. In this study, to enhance bone formation ability, we fabricated carbonate apatite-chitosan scaffolds (CA-ChS) with different content of carbonate apatite (CA) and evaluated CA-ChS from a standpoint of cell proliferation using MC3T3-E1.

MATERIALS AND METHODS : Chitosan scaffolds (ChS) which contained 200mg chitosan was fabricated by the following procedure. Two hundred mg chitosan (100D, YSK, Japan) was dissolved into 5ml of 2% acetic acid, shaked for 15 min, then neutralized with 15ml of 0.1M NaOH solution. After centrifugation at 1500rpm for 10 min, excess water was removed, and then the chitosan gel was packed into the molds (diameter: 5mm, height: 2mm). The molds were frozen at –80˚C for 2h and dried in a freeze dry machine for 24h for sponge body. The sponges were subjected to UV radiation for 2h. To fabricate CA-ChS, 10, 50, 100 and 200mg of 0.06M carbonate apatite (CA) were used. After neutralization, 10, 50, 100 and 200mg of 0.06M CA was added into the 200mg ChS. The structure of CA-ChS was observed by scanning electron microscope (SEM). Mouse osteoblast-like cell (MC3T3-E1) proliferation in this scaffold was investigated at 1, 7, 14, 21 and 28 days.

RESULTS AND CONCLUSION : Three dimensional porous structures of CA-ChS with CA powder attachment were clearly observed by SEM. Proliferated cell numbers in CA-ChS was significantly higher than those in ChS (control) at each stage of 1, 7, 14, 21 and 28 days ($p<0.05$). Under the limited results of this study, it may suggest that CA-ChS is a possible candidate for bone tissue engineering.

25-2

The aspects of osseointegration of the implants placed in the reinforced bone by using interconnected porous calucium hydoroxyapatite (IP-CHA)

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BACKGROUND AND HYPOTHESIS : The preservation of alveolar bone ridge is important for implant treatment. This study evaluated the aspects of osseointegration of the implants placed in the reinforced bone by interconnected porous calcium hydroxyapatite (IP-CHA).

DESIGN AND EXPERIMENTAL METHODS USED : This study was approved by the Research facilities Committee for Labpratpry Animal Science at Hiroshima University. Cylinder type IP-CHAs as a bone substrate was placed into six bone sockets at both sides of the femur of three male HBD Dogs. IP-CHA at the left side was a 24-week sample. After 12 weeks of IP-CHA placement titanium implant was placed into half side of the IP-CHA at the right femur. (A half portion of the implant was contacted to the reinforced IP-CHA as a half of the femur itself). IP-CHA was also placed into the another bone socket as a 12-week sample. After 12 weeks of implant placement, dogs were sacrificed and the bone tissues involved the implant and or not were obtained. The block without implant was decalcified and stained with HE. The block with implant was prepared as undecalcified specimens and stained with toluidine blue. Bone formation at cortical bone area of the femur was examined histologically and new bone formation and bone-implant contact ratio was measured histomorphometrically at 12 and 24-week samples.

ESSENTIAL RESULTS : New bone formation was observed in both of the host bone and IP-CHA sides. Histomorphometrically, bone formation area was superior in the IP-CHA 24-week sample rather than IP-CHA 12-week sample. From the result of BIC ratio, osseointegration was achieved at the surface between implant and IP-CHA. Osseointegration was obtained around the implant in reinforced bone by IP-CHA.

CONCLUSION : IP-CHA might be expected to be a possible bone substrate to reinforce bone quality for implant placement.

25-3

Development of implant/interconnected porous calcium hydroxyapatite complex

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BACKGROUND : We developed a novel implant as a complex of implant fixture and unique interconnected porous calcium hydroxyapatite (IP-CHA).

STUDY OBJECTIVE : The purpose of this study was to investigate bone reaction around this Implant/IP-CHA complex.

EXPERIMENTAL METHODS : Hollow cylinders of IP-CHA (outside diameter: 6mm, inside diameter: 3mm, height: 8.5mm) were obtained for this study. After tap and countersink preparations were done inside of the cylinder, titanium implant (diameter: 3.75mm, length: 8.5mm) was placed into the cylinder to fabricate Implant/IP-CHA complex *ex vivo*. These complex were then placed into the prepared bone socket (diameter: 6mm, depth: 8.5mm) in the femur of four HBD Dogs (weight: 20-25kg). Bone sockets were allowed to heal for 2, 3 and 6 months (n=3). As a positive control, implants were simply placed in the femur without any bone substrate. At 2, 3 and 6 months stage of healing period, implant stability quotients (ISQ) were measured by resonance frequency analyzer. Then, the animals were sacrificed and tissue blocks involved the complex were obtained. Undecalcified polishing specimens of IP-CHA involved the implant samples were prepared and stained with toluidine blue, which were observed by microscopy.

RESULTS : In complex group, ISQ values of 6 months (77.8±2.88) were significantly higher than the 2 months (47.4±11.4). Additionally, ISQ values of 6 months complex group were not significantly differences with 6 months control group (81.0±3.28). Microscopically, although newly formed bone was partially contacted to implant surface at 2 and 3 months complex groups, immature connective tissues including capillary angiogenesis dominantly existed around implant. Meanwhile, in 6 months complex group, newly formed bone was contacted most surfaces of the implant.

CONCLUSION : Within the limited results of this study, the Implant/IP-CHA complex might be able to be expected to reinforce both bone reconstruction and implant placement.

25-4

Fungicidal effect of novel antimicrobial peptides derived from *Lactobacillus rhamnosus* L8020

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BACKGROUND : *Candida albicans* and related *Candida* species are the most common opportunistic fungal pathogens encountered in the dentistry. Oral carriage of *Candida* and risk of fungal infection increase remarkably in patients with diseases, such as diabetes, immunosuppression and xerostomia.

Peptide antibiotics are considered a new class of antifungal agents. Recently, we determined the bacteriocin-like substances and encoded DNA by *Lactobacillus rhamnosus* L8020. At least two bacteriocin-like substances, kog 1 and kog 2, were produced by *Lactobacillus rhamnosus* L8020, both of which show the wide spectra and successfully killed *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis* and *Candida* spp. at or less than 0.39μM.

OBJECTIVE : The aim of this study was to evaluate fungicidal effect of two bacteriocin-like substances, kog 1 and kog 2 and compare with that of cationic mammalian antimicrobial peptides.

MATERIALS AND METHODS : Fourteen oral isolates, belonging to *Candida albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei* were used in the study. The antifungal effects of five peptides, *i.e.* kog 1, kog 2, histatin-5, JH8194 and lactoferricin B were examined according to the method of Nikawa et al (2004) with some modifications. The assays were carried out with quadruplicate samples and data were analyzed by ANOVA and multiple range tests.

RESULT : *Candida species*, other than *C. glabrata* were sensitive to five peptides used. Kog 1 and kog 2 ehxibited excellent fungicidal activities as well as other peptides used.

CONCLUSION : Both kog 1 and kog 2 exerted candidacidal activities against not only *C. albicans* but also non-albicans *Candida* species other than *C. glabrata*, suggesting that the new bacteriocin might be potent therapeutic agents for oral candidosis.

25-5

Expression and function of Retinoic acid-inducible gene-I (RIG-I) in oral keratinocyte and fibroblast

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BACK GROUND : The retinoic acid-inducible gene I product (RIG-I) has been identified as a cellular sensor of RNA virus infection resulting in anti-virus type I interferon induction. However, its expression and biological function in oral mucous membrane are not known. Here, we hypothesized RIG-I in oral fibroblasts and keratinocytes participate in host immune response to RNA virus infection. To prove this hypothesis, we examined the regulation of type I interferon and inflammatory chemokines through RIG-I in immortalized oral keratinocytes and fibroblasts.

MATERIAL AND METHODS : Total RNAs were extracted from immortalized oral keratinocytes (RT7), fibroblasts (GT1) and buccal epithelial cells from healthy volunteers. Single-stranded cDNA for a polymerase chain reaction (PCR) template was synthesized by reverse transcriptases. RIG-I expression patterns of these cells were examined using RT-PCR and Immunofluorescence staining. Induction of type I interferon (IFN-α, β) and inflammatory chemokines (CXCL10, CCL20, and IL-8) from RT7 and GT1 exposed to RIG-I ligand (Poly: IC/LyoVec complexes; Invivogen,Inc) were investigated by real-time RT-PCR and enzyme-linked immunosorbent assay (ELISA). In addition, phosphorylation of STAT1 and IRF3 induced by RIG-I ligand were analyzed by western blotting.

RESULT : RIG-I and Toll-like receptor 1-9 mRNA expressions were found in RT7, GT1 and buccal epithelial cells from healthy volunteers. Immunofluorescence analysis revealed the expressions of RIG-I in cytoplasm. RIG-I ligands caused an increase in mRNA levels of IFN-α, β, CXCL10 and IL-8 in RT7 and GT1. Small interfering RNA (SiRNA) for RIG-I and JAK/STAT inhibitor, AG490 suppressed RIG-I ligand-induced CXCL10 expressions in both cells. Furthermore, RIG-I ligand increased the level of STAT1 and IRF3 phosphorylation.

CONCLUTIONS : RIG-I in oral keratinocytes and fibroblasts may have important roles in the immune response in the regulation of type I interferon and inflammatory chemokines through the STAT1 and IRF3 signalling pathway.

25-6

CaCC activity, localization and stability of TMEM16E

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OBJECTIVES : TMEM16E has been found to be mutated in Gnathodiaphyseal dysplasia (GDD). TMEM16E also has been reported to be a member of TMEM16 family containing 8 transmembranes (TM) in topology. Recently, TMEM16A and TMEM16B were identified as the calcium activated chloride channel (CaCC) molecules. In this study, we determined if TMEM16E has CaCC activity and analyzed functional domains of TMEM16E.

METHODS : The GFP tagged proteins were stable expressed in culture cell lines and analyzed CaCC activity and localization. We also made chimeric TMEM16A/E such as AEA which was produced by substituting fragment from TM5 to TM6 of TMEM16A which encodes putative Cl- ionic pore domain, for that of TMEM16E.

RESULTS : TMEM16A has remarkable CaCC activity but neither TMEM16E nor AEA have the same CaCC activity when they were expressed in 293T cells. TMEM16E protein has lower protein stability while TMEM16A was sorted into plasma membrane with good protein stability. By analyzing the chimeric proteins, we found that head part of TMEM16E is important for protein stability because EAA, EEA have low stability similar with TMEM16E, contrary to AEE, AEA, AAE and TMEM16A which have the same good stability. TMEM16A altered its localization from the plasma membrane to the intracellular membranous compartment by the AAE chimera.

DISCUSSION : TMEM16E might have non-redundant function to CaCC to cause GDD. Our results suggest the protein stabilization system for TMEM16E could be supplied specially in the affected tissues (i.e. both jaw, limb long bones) in a GDD patient.

25-7

RHAMM induces proliferative activities of cementifying fibroma cells

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OBJECTIVES : Human cementifying fibroma is a benign fibro-osseous neoplasia in which normal bone is replaced with fibrous connective tissue containing abnormal bone or cementum. However, molecular mechanisms associated with proliferation and differentiation of cementifying fibroma remains poorly understood. We have found that the receptor for hyaluronan-mediated motility (RHAMM) was highly expressed in immortalized human cementifying fibroma cells (HCF) by microarray analysis. Therefore, biological behavior and role of RHAMM has been investigated in HCF cells.

METHODS : Reverse transcription-polymerase chain reaction (RT-PCR), western blot analysis and immunofluorescence microscopy were performed to examine RHAMM expression. Mineralization of HCF was examined by Alizalin red staining. To evaluate the contribution of RHAMM to the growth activity, we performed siRNA-mediated knock down for the elimination of RHAMM in HCF.

RESULTS : RHAMM knockdown inhibited the growth of HCF. The percentage of G2/M phase accumulation was reduced in RHAMM knockdown cells compared to control cells. These results indicated that RHAMM was involved in G2/M phase accumulation and play an important part in cell proliferation in HCF. RHAMM was necessary for ERK phosphorylation and could associate with ERK. Increased phosphorylation of ERK in the presence of hyarulonan (10μg/ml) were inhibited by RHAMM knockdown. The results suggested that RHAMM was also essential for hyarulonan-induced ERK activation. Interestingly, immunofluorescence microscopy showed that RHAMM moved to the nucleus immediately and associated with TPX2 at the centrosome in response to hyarulonan. Moreover, Aurora A kinase phosphorylation was increased in the presence of hyarulonan and blocked by RHAMM knockdown. Our results raised the possibility that RHAMM/TPX2 interaction was essential for the activation of Aurora A.

CONCLUSIONS : Consequently, it has been proposed that RHAMM plays a pivotal role in growth mechanisms of HCF by activating ERK and Aurora A kinase in HCF.

25-8

Fabrication and basic properties of poly (DL-lactide-co-glycolide) containing carbonated apatite

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BACKGROUND : It has become important to develop effective material to be used as scaffolds for bone tissue engineering. We fabricated new three-dimensional (3D) scaffolds consisting of biodegradable poly (D, Llactide-co-glycolic acid) (PLGA) (75/25) with carbonate-apatite (CO_3Ap). The aim of this study was to evaluate this new scaffold concerning its basic properties and biocompatibility.

METHODS : The CO_3Ap-PLGA composite scaffolds were fabricated by the solvent casting-particulate leaching method. In brief, PLGA (75/25) was dissolved in 10% DMSO solution. The PLGA solution was mixed with sugar crystals (0.65-0.80mm) and CO_3Ap powder in CO_3Ap ratio of 1:0.5 [CO_3Ap-PLGA (2/1)], 1:1 [CO_3Ap-PLGA (1/1)]. Then, all the dispersion was cast into a mold. Afterward, it was frozen immediately at –18˚C. The polymer was precipitated in deionized distilled water (ddH_2O) and the sugar crystals were leached out of the CO_3Ap -PLGA mixture. After that, the produced scaffolds were dried for 2 days. The obtained scaffolds were observed with scanning electron microscopy (SEM), and measured for porosity, shrinkage. The compositional change in the CO_3Ap-PLGA irradiation was analyzed with the use of powder X-ray diffraction (XRD).

RESULT : XRD patterns of CO_3Ap-PLGA (2/1), CO_3Ap-PLGA (1/1) was similar to those for the powder phase of PLGA and CO_3Ap-PLGA for comparison. Also, architectures of apatite was't made a transformation by the solvent casting-particulate leaching method. The average porosities of PLGA, CO_3Ap-PLGA (1/1) and CO_3Ap-PLGA (2/1) were 96.4%, 94.5%, and 89.1%, respectively.

In the shrinkage test, the mass reduction of PLGA was significantly larger than those of CO_3Ap -PLGA, and The structure of PLGA scaffolds began to collapse in 4weeks, However, those of CO_3Ap-PLGA composite didn't collapse until 6weeks

CONCLUSION : It was suggested that CO_3Ap -PLGA was more useful than PLGA in bone tissue engineering.

25-9

Immobilization of antimicrobial agent Et-QAC on the surface of denture acrylics and its evaluation of anticandidal activity

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BACKGROUND : Microbial plaque, including *Candida albicans*, on the denture fitting surface is thought to be an important etiologic factor in the pathogenesis of denture induced stomatitis. The importance of the control of biofilm on denture has been widely accepted. Therefore, chemical cleansing is recommended for denture plaque control. However, even if we cleaned denture by use of denture cleansers, biofilms will be formed at an early periods under the dairy use.

OBJECTIVE : The aim of our study were: first to investigate the effect of pretreatment on immobilization of oragnosilane quarterly ammonium salts (Et-QAC) on the acrylic surface, and second to examine the antimicrobial activity and the removal ability of biofilm by Et-QAC solution.

MATERIAL AND METHOD : *Candida albicans* GDH 19, an oral isolate obtained from the routine microbiology services of the Glasgow Dental Hospital and School, Glasgow, UK, was used in this study. Two commercial denture cleansers and oragnosilane quarterly ammonium salts (Et-QAC) solution were used in this study. The date was analyzed by ANOVA and multiple range test

RESULT : The results have shown that the pretreatment with ozone water, plasma and microwave irradiation were effective to immobilize Et-QAC on acrylics. In addition, Et-QAC solution has shown higher antifungal effects as well as the excellent removal ability against *Candida* biofilm as compared with commercial denture cleansers.

CONCLUSION : Et-QAC treatment significantly reduced the initial adherence or colonization of *C. albicans*. It was suggested that Et-QAC have a potential to a cleanser which will provide the antimicrobial barrier on denture surface.

25-10

Application of immobilizing disinfectant to the hydroxyapatite

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PURPOSE : The purpose of this study was to research bactericidal effects of mouth washes which can care oral health for people who can't do mechanical tooth cleaning.

METHODS : *Streptococcus mutans*. was used in this study.

In the first place, we compared the bactericidal effects of commercial mouth washes on planktonic bacterial cells with that on biofilm cells. In the second place, we evaluated the antibacterial effects of the quartery ammonium salt immobilized on hydroxylapatite (Etak-treated HAP). The biofilm assay was conducted according to the method described by Nikawa et al. (Microbial Ecol9, 35-48, 1996) on hydroxyapatite surfaces.

RESULT AND CONCLUSIONS : The antimicrobial activities of commercial mouth washes against planktonik cells varied depending upon the products, though all products have shown little effect against the biofilm cells. Thus to inhibit the microbial biofilm formation, the quartery ammonium salt was immobilized on HAP, and the results demonstrated the excellent antimicrobial effect of Etak-treated HAP on biofilm formation of *Streptococcus mutans*.

25-11

Evaluation of physical properties of commercially artificial saliva

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OBJECTIVE : Saliva is known to have several distinct functions including lubrication, mucosal integrity, and antimicrobial action. Therefore, reduction of salivary flow usually leads to dysfunctions of speech, chewing or swallowing, and further to impaired taste sensation. Artificial saliva are often prescribe to improve the symptoms caused by the dehydrated oral mucosa or dry mouth. The aims of this study were to evaluate viscosity, moisturizing ability and organoleptic examination of 13 commercial artificial saliva and 3 prototypes.

MATERIALS AND METHODS : Thirteen-commercial products of artificial saliva were purchased and used in the present study, and three prototyped artificial saliva were provided by JEX Co., Ltd. (Osaka, Japan).

The viscosity of the of each products was evaluated for three times by the use of Sine-wave Vibro Viscometer at the room temperature 25˚C and range 4-60˚C.

To evaluate the ability of moisturizing, 0.1ml of each sample was applied on the forearm of the volunteer, who stayed for 30 minutes at 20˚C in advance and the moisture value of skin was measured five times for each sample at 0, 5, 10, 15, and 20 minutes after application, using an oral moisture checking device.

The taste, feeling in the mouth, flavor and wettability of each product were quantified by the use of Visual Analogue Scale (VAS).

RESULTS : The viscosity varied to a greater extent, depending upon the products and the viscosity of the all products decreased accompanied with the increase in temperature. The potential to moisturize was the greatest with Fit angel gel type, followed by prototype. The taste, feeling in the mouth, flavor and wettability varied depending on either the individuals or products.

CONCLUSION : Commercial artificial saliva should be chosen depending upon, their viscosity, moisturizing ability and favor of taste, feeling in the mouth, flavor and wettability.

25-12

Evaluation of antibacterial activity of commercial artificial saliva

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BACKGROUND : In recent years, xerostomia patients have increased in young individuals as well as older individuals. Xerostomia may be caused by age-related atrophy of salivary glands, a side effect of a radiation therapy, and some kinds of medications. It has been pointed out that oral environment deterioration by hyposalivation has risks to cause fungal infections and an increase in the risk of caries, root caries and/or periodontal disease, and respiratory infection by aspiration of oral bacteria or fungi. Artificial saliva are known as one way to improve the symptoms of xerostomia. Commercially available artificial saliva products are mainly divided into two types, *i.e.* gel type and liquid type. To prevent the bacterial or fungal growth, antimicrobial agents, such as cetylpyridinium chloride (CPC) or Hinokitiol were incorporated into some products. However, there is little information available on antimicrobial activity of these products.

STUDY OBJECTIVES : The purpose of this study was to evaluate antibacterial activity of 13 commercial artificial saliva and 3 prototypes.

METHODS : The growth and biofilm formation of *Candida albicans* and *Streptococcus mutans* were studied by measuring pH or optical density of the media and by the use of adenosine triphosphate (ATP) analysis.

RESULTS : PrototypeA, B and Viva jellwet showed the greatest antibacterial activity against the growth of *C. albicans* and *S. mutans*, while, prototypeC, as well as Viva jellwet inhabited *C. albicans* biofilm formation effectively. BioXtra and Fit angel, Aqua mucus significantly inhibited the biofilm formation, though these products had no antimicrobial activity.

CONCLUSIONS : The results suggested that the antibacterial activity of commercial artificial saliva varied depending up on the product and that all of 3 prototypes and Viva jellwet into which CPC was incorporated, showed the highest antibacterial activity.

25-13

Effects of biomimetic diamond-like carbon coated titanium on the differentiation of osteoblast and osteoclast cells

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BACKGROUND : Osseointegration is the creation of a direct structural and functional connection between living bone and the surface of a dental implant. At the interface, continuous and dynamic remodeling, replacement of old bone, repair of fatigue-damaged foci and maintenance of interface structural integrity occurs. Successful bone remodeling in dental implants requires balanced activities of both osteoblasts and osteoclasts. Some reports suggest that a surface modification method on Ti that decreases osteoclast activity could increase osteoblast activity, leading to enhanced osseointegration.

biomimetic diamond-like carbon (DLC) is a durable and compatible biomaterial. We investigated effects of biomimetic DLC coated titanium on the differentiation of osteoblast and osteoclast cells.

MATERIALS AND METHODS : Pure wrought titanium (Ti) disks were used in the experiments. Disks with biomimetic standard DLC (DLC-A), biomimetic DLC-coated Ti with carboxyl group (DLC-B) and biomimetic DLC-coated Ti with amino group (DLC-C) were deposited by using an ionized deposition apparatus for the DLC films.

Effects of Runx2 and Type I collagen on mRNA expression were analyzed by using osteoblastic cell lines, MC3T3-E1 and effect of TRAP mRNA expression were analyzed by using osteoclastic cell line RAW264.7 exposed to RANKL stimulation. They were cultured on untreated Ti (control), DLC-A, DLC-B, and DLC-C.

RESULTS : DLC-coated Ti enhanced the mRNA expressions of the osteoblast differentiation markers including Runx2 and type I collagen, in MC3T3-E1. In contrast, DLC-coated Ti inhibited the mRNA expressions of osteoclast differentiation marker; TRAP, in RAW264.7 exposed to RANKL stimulation.

DISCUSSION : These results, taken together, suggested that DLC-A might enhance bone formation around dental implants.

25-14

The effect of bacteriocins derived from *Lactobacillus rhamnosus* against oral pathogens

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BACKGROUND : Dental caries is known as one of the most important disease in dentistry, since the dental caries is a major cause of tooth loss or failure of crown/bridge works.

OBJECTIVE : Antimicrobial effects of two bacteriocins, *i.e.* kog1 and kog2, derived from *Lactobacills rhamnosus* (*L. rhamnosus*) KO3 against cariogenic bacteria was examined in the present study.

MATERIALS AND METHODS : *Streptococcus mutans* Ingbritt (*S. mutans*) was used in the study, and human beta-defensins-2,-3, human Lactoferricin, bovine Lactoferricin, and Histatin 5 were employed to compare the antimicrobial activity with kog1 and 2. The antimicrobial effect of kog1, kog2, was examined according to the method described by Nikawa et al (2004). Briefly, 20μl of the bacterial suspension was mixed with equal volume of 1mM sodium phosphate buffer (Na_2HPO_4/NaH_2PO_4) containing the test peptide, ranged from 0.20 to 50.00μM and was incubated for 90 min at 37°C with shaking. As controls, 20μl of the bacterial suspension was mixed with 20μl of peptide free 1mM phosphate buffer. The reaction was stopped by adding 360μl of Yeast Nitrogen Base medium (Difco) and 40μl of the sample was inoculated onto Mitis Salivarius Agar (Difco) plates, incubated for 48h at 37°C, and the colony forming units (CFUs) in the test and control specimens were quantified.

RESULTS : The antibacterial effects of both kog1and kog2 were excellent as compared with the cationic antimicrobial peptides derived from mammalian cells.

CONCLUSION : The cationic bacteriocins derived from *L. rhamnosus* L8020, have shown the excellent antimicrobial effects against cariogenic bacteria.

25-15

Bacteriocins of *Lactobacillus rhamnosus* L8020 inhibits *Porphyromonus gingivalis* lipopolysaccharide-induced pro-inflammatory cytokine secretion in murine macrophage-like RAW264.7 cells

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BACKGROUND AND RATIONALE : Periodontitis is a major oral disease that causes destruction of periodontal tissue and finally loss of teeth. In recent years, it has been appeared that periodontitis is a risk factor of systemic disease such as cardiac disease, cerebrovascular disease and diabetes.

Lactobacillus rhamnosus KO3 strain is a kind of lactobacillus that is isolated from oral cavity of caries-free volunteer. We have already confirmed that kog1 and kog2, bacteriocin of *Lactobacillus rhamnosus* KO3 strain, have excellent antibacterial effect.

HYPOTHESIS OR STUDY OBJECTIVE : The purpose of this study was to evaluate of bacteriocins derived from *Lactobacillus rhamnosus* L8020, *i.e.* kog1 and kog2, on the expression and secretion of pro-inflammatory cytokine (TNF-α) and chemokine (CCL2) in *Porphyromonus gingivalis* lipopolysaccharide (*P.g.*-LPS)-activated macrophage-like cells.

DESIGN AND EXPERIMENTAL METHODS USED : The RAW264.7 cells, a murine macrophage cell line, were used this study. Theeffect of kog1 and kog2 on the viability of RAW264.7 macrophage cells was examined by MTS assay. The induction of proinflammatory cytokine and chemokine in RAW264.7 cells by Pg-LPS in the presence or absence of the bacteriocins were analyzed by real-time quantitative RT-PCR and ELISA.

ESSENTIAL RESULTS : MTS assay showed that kog1 and kog2 (0, 1, 5, 10 and 20μM) had no significant effects on the cell viability of RAW264.7 cells (ANOVA: $p>0.05$). Kog1 and kog2 suppressed *P.g.*-LPS-induced TNF-α and CCL2 expressions and protein secretions in a dose dependent manner (ANOVA: $p<0.01$).

CONCLUSION : Kog1 and kog2 inhibited *P.g.*-LPS-induced expression and secretion of TNF-α and CCL2 in macrophage-like cells. These results, taken together, suggested that kog1 and kog2 could possibly be a very good therapeutic agent candidate for treating periodontal disease.

25-16

Calcium phosphate nucleation ability on the Titanium surface modification by self assembled monolayers

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INTRODUCTION : Commercially pure titanium (cp Ti) is widely used in dental implantology. However, being bioinert, it is only passively integrated in bone. With the objective of increasing the chemical interaction between the cp Ti and bone tissue, several alkylphosphonic acids with carboxyl group were firstly synthesized onto titanium surface via self assembled monolayer (SAM) technique, and then were immersed into simulated body fluid (SBF) to evaluate the calcium phosphate nucleation ability.

MATERIALS AND METHODS : The bare polished Ti disks and the grafting of three alkylphosphonic acids with carboxyl group (*3-phosphonopropionic acid* (3-PA), *6-phosphonohexanoic acid* (6-PA), and *16-phosphonohexadecanoic acid* (16-PA)) on the disks were characterized with X-ray photoelectron spectroscopy (XPS). Then, each sample was immersed in 50mL SBF and kept in 37°C. The chemical and morphological changes on the Ti surface were determined by XPS and scanning electron microscopy (SEM), respectively.

RESULTS AND DISCUSSION : After introduction of PAs, P2p was observed by XPS, which indicated that the PAs were successfully introduced onto the surface of Ti. Moreover, ratio of P/Ti increased from control to 16-PA. It supposed that the immobilized amount of PA molecules increased 3, 6, and 16-PA, respectively. After SBF immersion, Ti2p was detected on the 3-PA and control but not on the 6-PA and 16-PA modified titanium surfaces. Meantime, Ca2p was strongly detected on the 6-PA and 16-PA, whereas weakly on the 3-PA and control. From SEM images, the small and fine calcium phosphonate particles were densely deposited on the 16-PA and 6-PA surfaces whereas almost no particles deposited on the 3-PA and control, which also indicated that immobilized amount of PA was positively correlated with calcium phosphonate nucleation ability.

CONCLUSIONS : Compared with 3-PA and control, 16-PA and 6-PA modified Ti surface is easier to induce calcium phosphate nucleation.

25-17

Anti-M3 muscarinic acetylcholine receptor antibody causes aquaporin-5 dependent hypo-secretion of saliva in a mouse model of Sjögren's syndrome

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Sjögren's syndrome (SjS) is a chronic autoimmune disease characterized by hyposecretion of tears and saliva from lacrimal and salivary glands. Several recent studies have reported that the levels of IgG autoantibodies reacting to M3 muscarinic acetylcholine receptor (M3R) are increased in SjS patient serum. Importantly, it has been found that M3R expressed on the glandular cell surface is engaged in the water secretion. However, the pathophysiological role of anti-M3R autoantibodies in SjS remains unclear. Therefore, the aims of this study were to 1) develop an anti-M3R monoclonal antibody (mAb) and 2) evaluate its patho-physiological impact on the development of SjS. BALB/c mice were immunized with synthetic M3R peptide. Splenocytes isolated from the immunized mice were fused to mouse myeloma cells to generate hybridoma cells that produce IgG class mAb to M3R. ELISA assay showed that the purified anti-M3R mAb reacts to the extracellular second loop among a total three loops of M3R. The binding specificity to M3R expressed on glandular epithelial cells was confirmed by immunohistochemistry and immunoblotting. Then, anti-M3R mAb (10mg/0.5ml PBS/mouse) or control PBS (0.5ml/mouse) was adoptively transferred to mice (i.v). At Day-14, the anti-M3R mAb-treated mice, compared to control mice, showed significantly lower secretion of saliva. Most importantly, aquaporin 5 (AQP5), a water transportation channel, is normally expressed on apical sides of the gland acini, while anti-M3R mAb-treated mice showed aberrant expression of AQP5 at the basement membrane area of gland acini. Moreover, in the following proof-of-concept *in vitro* experiment, anti-M3R mAb inhibited the carbachol (M3R agonist)-induced translocalization of AQP5 from cytoplasm to cell membrane in human salivary gland cells. Taken together, these findings demonstrated that auto-anti-M3R IgG antibody interrupts the translocalization of AQP5 in salivary gland, which results in inhibiting salivary flow, thus indicating the pathophysiological role of anti-M3R IgG antibody in SjS.

25-18

F-spondin promotes the differentiation of human periodontal ligament cells

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BACKGROUND : Cementum plays an important role in the connective tissue attachment to the root surface. However, detailed mechanism of cementum formation has not yet been clarified. We previously established human cementoblast-like cell lines (HCEM) and human periodonatl ligament cell lines (HPL) by infection of hTERT gene (Bone, 2006). Using these cell lines, we showed that F-spondin is a gene with the highest fold expression in HCEM. F-spondin is a secreted adhesion molecule originally isolated from the embryonic floor plate of vertebrates. Expression of F-spondin was reported in various tissues and cells; floor plate epithelium, chondrocytes and so on. Little is known about a role of F-spondin in periodontal tissue.

OBJECTIVE : We clarify the role of F-spondin in differentiation of cementoblasts.

METHODS : HPL-F-spondin cells (HPL-SPON1) was transfected with retroviral plasmid encoding a rat F-spondin cDNA provided by Dr. Klar (Hebrew University) into HPL. F-spondin expression of HCEM was knock downed by siSPON1. We examined expression of mineralization associated mRNAs and proteins by alkaline phosphates (ALP) activity, collagen assay, RT-PCR and western blot in these cells.

RESULTS : RT-PCR indicated higher expression of ALP, osteocalcin (OCN) and bone sialoprotein (BSP) and lower expression of type I collagen (COLI) in HPL-SPON1 than in HPL. ALP activity and collagen assay showed same results. And HPL-SPON1 increased MMP13 mRNA expression. Moreover, BMP7 and phosphorylated Smad1/5/8 were detected in HPL-SPON1. The knock-down by siSPON1 decreased mRNA expression of F-spondin, RUNX2, BSP and BMP7 in HCEM.

CONCLUSION : F-spondin promotes expression of mineral associated genes and regulates differentiation of cementoblast through BMP7 expression. Therefore, inducing F-spondin in HPL might be a potent regenerative therapy for cementum and the connective tissue attachment to the root surface.

25-19

Long-term cryopreservation of rat MSCs by use of a programmed freezer with a magnetic field

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Mesenchymal stem cells (MSCs) can be used for the regeneration of various organs and tissues. However, there has been little information about cryopreservation of MSCs. The purpose of this study was to evaluate the influences of cryopreservation on MSCs by use of a programmed freezer with a magnetic field (CAS freezer) which is developed to prevent cell damage caused by intracellular ice crystal formation.

MSCs were isolated from bone marrow of rat femora. The cells were frozen with a cryoprotectant, 10% DMSO or 20% trehalose by a CAS freezer for 7 days at –150˚C. Immediately after thawing, the number of surviving cells was counted and the cells were cultured; cultured cells were examined after 48 h. Results indicated that a 0.01mT magnetic intensity, a 15-min hold-time, and a plunging temperature of –30˚C created the greatest survival rate of MSCs. Moreover, after treated with bone differentiation medium, alizarin positive reaction, large amount of calcium deposition, and greater alkaline phosphatase activity were shown in both control and cryopreserved groups. After the fat differentiation, Oil Red O dye positive reaction was found in both control and cryopreserved groups.

It is shown that a CAS freezer is available for long-term cryopreservation of MSCs and that the cryopreserved cells can be applied to tissue regeneration medicine.

25-20

An establishment of jaw cleft treatment with bone regeneration by use of mesechymal stem cells derived from bone marrow

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BACKGROUND AND STUDY OBJECTIVE : Autogenous iliac bone grafting has been successfully employed for the closure of bone defects in patients with cleft lip and palate (CLP). However, the related surgical procedures require substantial surgical invasion and cause subsequent psychological stress. Thus, an application of mesenchymal stem cells (MSCs) derived from bone marrow to bone regeneratoion has been suggested. In addition, tooth movement to the regenerated site is required during the treatment of CLP patients, therefore, it is essential that regenerated bone has physiological metabolic function and with appropriate resorption of scaffold. The purpose of this study is to investigate the localization of MSCs after transplantation *in vivo* and the interaction between MSCs and carbonated hydroxyapatite (CAP) scaffold.

EXPERIMENTAL METHODS USED : MSCs extracted from iliac bone, were cultured and labeled by fluorescent nanoparticle. The MSCs were transplanted with CAP scaffold into artificial bone defects created in bilateral canine region of the maxilla in 3-month-old beagle dogs. The bone regeneration and the absorption of CAP were evaluated by X-ray images and histological examination.

ESSENTIAL RESULTS : Bone regeneration by MSCs and CAP scaffold in artificially created bone defects of beagle dog was observed. In addition CAP with MSCs was absorbed more rapidly than CAP alone. Histological analysis revealed that fluoresceinated osteocytes differentiated from MSCs were observed in regenerated bone and tartrate resistant acid phosphatase (TRAP) positive cells were observed on the surface of CAP.

CONCLUSIONS : The maxillary bone defects were successfully regenerated by the transplantation of MSCs and CAP scaffold, suggesting that applications of this method may be available as a new therapeutic tool for CLP.

25-21

Amelogenin-guided biomineralization of hydroxyapatite crystal on tooth enamel

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BACKGROUND AND RATIONALE : The enamel mineral tissue is developed from a layer of soft protein matrix secreted by a layer of epithelial cells, the ameloblasts. Mature tooth enamel becomes a highly mineralized extracellular tissue, consisting of approximately 96% minerals and only 4% organic materials and water. The dominant enamel matrix protein is amelogenin, which constitutes more than 90% (w/w) of the proteins in the enamel protein matrix, and has been considered to play an essential role in the control and modulation of enamel crystal growth.

STUDY OBJECTIVE : The purpose of this study was to investigate the *in vitro* amelogenin-guided induction of hydroxyapatite crystal on the surface of tooth enamel.

DESIGN AND EXPERIMENTAL METHODS : Recombinant human full-length amelogenin (rh174) was synthesized and purified. The surface of extracted teeth was treated with solution containing 0-4mM calcium nitrate, 0-32mM potassium phosphate, and 0-5mg/ml rh174 in 1.7-mL siliconized tubes for 16 hr at 25˚C. The crystal formation on tooth enamel surface was evaluated using digital binocular wide-field dissecting microscope and atomic force microscope (AFM) in tapping mode with silicon tips. Roughness (Ra) of enamel surface before and after the treatment was quantified using AFM and VN viewer software.

ESSENTIAL RESULTS : The crystal formation on the surface of tooth enamel was shown after the treatment with rh174, whereas no crystal induction was observed in the control group treated with solution not containing rh174. The surface roughness (Ra) was decreased significantly by the treatment with rh174 as compared to the control group.

CONCLUSION : Amelogenin-guided biomineralization of hydroxyapatite crystal on the surface of tooth enamel leading to decrease in the surface roughness of enamel was shown, suggesting it may become a candidate as medical remedy to treat damaged enamel crystal using amelogenin.

25-22

Immunohistochemical study of periodontal tissue regenerative process by BDNF

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OBJECTIVES : Brain-derived neurotrophic factor (BDNF), one of the neurotrophin family, affects neural development, survival and repair. Furthermore, BDNF is involved with hard tissue regeneration. We have already revealed that BDNF/hyaluronic acid (HA) complex enhances periodontal tissue regeneration *in vivo*. Periodontal tissue regeneration process may work as a cascade. In the cascade, adequate molecules and cells work at adequate timing and duration to regenerate tissue. In this study, we have performed histological analysis of early stage of periodontal tissue regeneration by BDNF to reveal the cascade.

METHODS : The class III furcation defects were created surgically at the second, third and fourth premolars in female beagle dogs. The cementum faced to the defects was removed and then impression materials were placed in the defects to induce inflammation. BDNF (50μg/ml)/HA complex was applied into the defects. Two weeks after the application, periodontal tissue regeneration was histologically evaluated. The localization of TrkB (BDNF high-affinity receptor), osteopontin (OPN) and proliferating cell nuclear antigen (PCNA)-positive cells was evaluated by immunohistochemistry.

RESULTS : Two weeks after the BDNF application, periodontal tissue regeneration was insufficient, but invasion of epithelial cells to defects was not observed. TrkB -positive cells were detected on the denuded root surface, around the regenerating alveolar bone and in the soft connective tissue of regenerating periodontal tissue. PCNA-positive cells mainly observed around the regenerating alveolar bone and in the soft connective tissue of the defect. Regenerating alveolar bone was also immunoreactive for OPN.

CONCLUSION : TrkB positive cells quickly responded to BDNF/HA complex and started to proliferate and differentiate for the periodontal tissue regeneration within 2 weeks. However, detailed cell regulation on the dentin surface, around the regenerating bone and in the soft connective tissue is not still clear.

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Isolation and osteogenic differentiation of jaw bone marrow derived mesenchymal stem cells in beagle dogs

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As a result of previous clinical study, there are some issues to transplant mesenchymal stem cells (MSC) for regeneration of periodontal tissue. MSC were aspirated from bone marrow of ilium in the study, however, puncture into ilium is high risk and much painful for donor. To reduce risks and discomfort, we selected jaw bone as MSC source. And all process can be practiced and managed by dentists. In this study, we isolated MSC from jaw bone of beagle dogs and examined the potential of osteogenic differentiation of these cells.

Eight beagle dogs (female, 12~20 month old) were used in this study. We selected the furcation of both right and left side of the lower first molar as aspirating sites of bone marrow. Under general and local anesthesia, the cortical bone was exposed punctured by a steel bar (diameter 1.8mm). A 16G indwelling needle with a 10ml syringe was connected to the hole and bone marrow was aspirated. The bone marrow aspirate was mixed with α-MEM (containing 10% FCS, penicillin, streptomycin) and incubated for one week. Then blood corpuscle and cells unattached to the dish were washed out and the flesh culture medium was supplied. The colony formation of cells was valuated at 10 days. After collected cells were frozen and stored, these cells were re-cultured in osteogenic medium to evaluate osteogenic differentiation ability.

There was no perforation into root of tooth or mandible canal. Bone marrow aspirate was able to collected over 2ml in all tested sites. The colony formation was confirmed in 10 samples out of 16 samples (62.5%). Osteogenic differentiation of these cells was observed in all of 10 samples as well as MSCs from ilium. These results suggested that cells aspirated from jaw bone might be useful in transplanting for regeneration of periodontal tissue.

25-24

The inhibitory effect of irsogladine maleate against Toll-like receptor 2 induction mediated by *Porphyromonas gingivalis* in human gingivalis epithelial cells

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BACKGROUND : Periodontitis is caused by the interaction between bacterial challenge and host response. *Porphyromonas gingivalis* (*P.g*) is a gram-negative obligate anaerobe and the crucial etiologic agent in the initiation and progression of periodontitis by producing some pathogenic factors. *P.g* whole bacteria and its components, outer membrane proteins (OMPs), lypopolisaccharide (LPS) and gingipain, are known to be Toll-like receptors (TLRs) ligand. TLR2 is one of the receptors which expresses on the surface of gingival epithelial cells. Irsogladine maleate (IM) is the anti-inflammatory agent, clinically used as an anti-gastric ulcer. Previously we reported the inhibitory effect of IM against IL-8 induction mediated by *Aggregatibacter actinomycetemcomitans* (*A.a*) in human gingival epithelial cells. However, the regulation of bacterial recognition protein, such as TLRs and NODs by IM is unclear. Therefore, in this study, we examined the inhibitory effect of IM on TLR2 mRNA expression stimulated by *P.g* in human gingival epithelial cell (HGEC).

MATERIALS AND METHODS : Human gingival immortalized epithelial cell (OBA-9) were cultured in KG2 medium (KURABO, Japan). The confluent culture of OBA-9 were pretreated with IM (1μM) for 30 min before *P.g* (10^8 CFU/mL) stimulation. After 6H stimulation, total RNA was extracted for real-time PCR to examine IL-8 and TLR2 mRNA expression. In order to determine the IL-8 production in the supernatant, ELISA was performed by using IL-8 ELISA Development Kit (Peprotech NJ, USA). Anti-CXCR1 antibody, anti-CXCR2 antibody and anti-IL-8 antibody were applied into the medium before *P.g* stimulation to determine the involvement of IL-8 in TLR2 mRNA induction. To observe IL-8 effect on TLR2 induction, OBA-9 were stimulated by recombinant IL-8 (1ng/mL and 0.5ng/mL) with or without IM.

RESULT : *P.g* contact showed the mRNA induction of TLR2 and IL-8 compared with no stimulation (5 folds and 30 folds, respectively). IL-8 production was also increased (3 folds) by *P.g* stimulation. The addition of recombinant IL-8 induced the TLR2 mRNA expression (2 folds). However, IM pretreatment inhibited mRNA expression of TLR2 mediated by *P.g* and IL-8 stimulation in HGEC. The application of anti-CXCR1 antibody, anti-CXCR1 antibody, and anti-IL-8 antibody inhibited the induction of TLR2 mRNA expression.

CONCLUSION : IM inhibited TLR2 induction in HGEC stimulated by *P.g*. This inhibitory effect of TLR2 might be mediated by IL-8 suppression.

25-25

Two cases of bisphosphonate-related osteomyelitis of the mandible with solid type periosteal reaction

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BACKGROUND : Bisphosphonates (BPs) are the drug that decreases bone turnover by inhibiting osteoclast activity. BPs have been widely used for the treatment of osteoporosis, Pagets disease, multiple myeloma, hypocalcemia of malignancy, and bone metastasis of solid cancers. In recent years, the incidence of osteonecrosis of the jaws as a complication in patients receiving bisphosphonates has been on the increase.

CASE REPORT : Two female patients were examined by periapical radiography, panoramic radiography and computed tomography. Case 1 was a 75 year old female with a large radiolucent lesion in the right mandibular molar area. She had been prescribed intravenous bisphosphonate for the treatment of breast cancer for last 3 years. Case 2 was a 84 year old female with a radiolucent lesion around the apical area of the lower left 3rd molar. She had been taking intraoral bisphosphonate for the treatment of osteoporosis for several years.

Panoramic and periapical radiographs showed osteolysis in the mandibular alveolar bone in both patients. Sclerotic changes were observed in the surrounding cancellous bone. Contrast computed tomography (CT) of the mandible showed a bony defect and a Solid type of periosteal reaction in both cases.

DISCUSSION : In our earlier study of osteomyelities of the jaws, (non-diffuse sclerosing osteomyelities), the lamellated-type periosteal reaction was observed in 22 patients out of 48. In case of 24 BRONJ patients the lamellated type of periosteal reaction was not found but the solid type of periosteal reaction was observed in 7 patients. So it might be suggested that solid type of periosteal reaction may be one of the diagnostic criteria of bisphosphonate-related osteonecrosis of the jaw.

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Adipose tissue inflammation and smoking synergistically suppress leptin expression in Japanese obese males: Potential mechanism of resistance to weight loss among obese smokers

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OBJECTIVE : The effect of smoking on leptin regulation is controversial. Additionally, recent series of studies indicated the critical role of macrophage migration in the establishment of adipose tissue inflammation. In this study, we aimed to see the effects of smoking and inflammation on leptin regulation both at cellular and epidemiological levels.

DESIGN : We compared the concentration of inflammatory markers and serum leptin levels among Japanese male subjects with or without smoking. Additionally, leptin and ICAM-1 gene expression was assessed in adipocytes co-cultured with or without macrophages in the presence or absence of nicotine and/or lipopolysaccharide (LPS).

RESULTS : In subjects with BMI below 25kg/m^2, both WBC counts and sICAM-1 levels are significantly higher in smokers than in non-smokers. However, leptin concentration did not differ according to smoking status. However, in subjects with BMI over 25kg/m^2, smokers exhibited significantly lower serum leptin level as well as higher WBC counts and sICAM-1 concentration as compared with non-smokers. Leptin gene expression was markedly suppressed in adipocytes co-cultured with macrophages than in adipocyte culture alone. Nicotine further suppressed leptin gene expression in co-cultures. ICAM-1 gene expression was markedly up-regulated in adipocytes co-cultured with macrophages when stimulated with LPS.

CONCLUSIONS : Adipose tissue inflammation appears to down-regulate leptin expression in adipose tissues. Nicotine further suppresses leptin expression. Thus, both inflammation and smoking diminish leptin effect in obese subjects. Therefore, obese smokers may be more resistant to weight loss than non-smokers.

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Age-related changes of sensory thresholds in the oro-facial region and the relationship of sensory thresholds with dysphagia

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BACKGROUND : Oral sensation plays an important role in modulation of mastication and impairment of oral sensation possibly cause delay or disappearance of the swallowing reflex leading to aspiration or dysphagia.

OBJECTIVE : The aim of this study was to determine the age-related changes of tactile and thermal sensitivity, and the relationship of the sensitivity to dysphagia.

SUBJECTS AND METHODS : A hundred and eighty-seven subjects (44 males and 145 females: age range 20 to 96 years) without history of neurological impairment and trauma or surgery in the head and neck were enrolled in this study.

First, tactile sensations were examined with Semmes-Weinstein monofilaments and two-point discriminator in the oro-facial region. Second, thermal sensations were examined with a Sammi thermal tester in the region. Third, four questionnaire investigations (Self-rating Depression Scale, Barthel Index, NM Scale, Questionnaires about dysphagia) were performed to know the levels of depression and dysphagia, ADL, and mental state. Then, the relationship of sensations in the oro-facial region with dysphagia was analyzed in the 65-year-old and more senior sublects.

RESULTS : In the Semmes-Weinstein test, the thresholds of eighties and nineties were significantly higher than those of twenties and thirties at the tip of the tongue and the buccal mucosa. In the two-point discrimination test, the thresholds of eighties and nineties were significantly higher than those of any other age group in all the sites tested. In the warm sensation test, the thresholds of eighties and nineties were significantly higher than those of sixties and seventies at the buccal mucosa. A statistical weak relationship was observed between dysphagia and the Semmes-Wesintein sensation at the anterior site of the palate.

CONCLUSIONS : This study indicated that the sensory thresholds in the oro-facial region increased with age, especially in the two-point discrimination test and that the threshold at the anterior site of the palate weakly related to dysphagia.

25-28

Inhibitory effect of bLF on LPS-induced crosstalk among TLR pathways in osteoblast

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Host immune responses to pathogenic factors of periodontal pathogens have a crucial role in the alveolar bone destruction in periodontal disease. The toll-like receptor (TLR) family of pattern recognition receptors is responsible for the immune responses against the pathogens. It is well accepted that lipopolysaccharide (LPS) with a strong capacity of cytokine-induction through TLR4 pathway plays a central role in the pathogenesis and progression of periodontal disease. Previously, we demonstrated that bovine lactoferrin (bLF) inhibited LPS-induced cytokines production such as TNFα in osteoblasts and reduced osteoclastogenesis via osteoblasts. Recently it has been reported that signaling crosstalk of TLR caused by LPS-TLR4 pathway is important for immune response of macrophages to pathogens. However, little is known about the crosstalk between TLR pathways in cytokine production by osteoblast and the effect of bLF on crosstalk caused by LPS-TLR4 pathway. In the present study, therefore, we investigated effects of LPS priming on TLR5- or TLR9-induced cytokine production as well as effects of bLF on the LPS priming consequence in osteoblast.

In this experiment, ST2 cells were used as osteoblastic lineage cells with supporting capacity of osteoclastogenesis. ST2 cells strongly expressed TLR4, but not TLR5 or TLR9. TLR4 stimulation by LPS caused remarkable up-regulation of TNFα mRNA level, whereas TLR5 and TLR9 stimulation with Fragellin and CpG-DNA, respectively, did not show any change of TNFα mRNA expression. LPS priming enhanced exression of TLR5 and TLR9 and up-regulated TNFα mRNA expression by them. Moreover, bLF pretreatment strongly inhibited TLR5 and TLR9 pathways-induced TNFα mRNA expression through down-regulation of TLR5 and TLR9.

As a result, we confirm that TLR4 signaling pathway controls other TLR expression levels and suggest that bLF decreases the sensitivity to pathogenic factors from bacteria and inhibits severe destruction of alveolar bone by controlling the crosstalk in TLRs pathways mediated by TLR4 pathway.

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EMT induction by humoral factors in oral squamous cell carcinoma cell

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BACKGROUND : Epithelial-mesenchymal transition (EMT) is involved in tumor progression and tumor cells with EMT phenotype exhibit high-invasiveness and metastatic ability. We have previously shown that forced expression of snail, a zinc finger transcriptional repressor and known as an EMT inducer, in squamous cell carcinoma (SCC) cells with epithelial phenotype induces EMT and gains their invasiveness *in vitro*. However, processes of the activation of EMT inducers are still unclear.

STUDY OBJECTIVE : On the hypothesis that EMT arises at cells in the invasive front of SCC tissues under the influence of tumor microenvironmental factors, we examined how humoral factors from the stroma exert potential influence to the EMT induction.

EXPERIMENTAL DESIGN AND METHODS : The EMT status in tumor cell was assessed by intracellular localization of E-cadherin and Vimentin detected by immunocytochemistry. After stimulations of humoral factors (*e.g.*, TGF-beta, TNF-alpha, PDGF-D) in SCC cell lines, mRNA and protein expressions of EMT inducers were monitored by RT-PCR and immunoblotting,

ESSENTIAL RESULTS : Simultaneous stimulation of the factors listed above generates a subset of EMT phenotype cells, which showed up-regulation and nuclear localization of snail or slug, and gain of the invasive capability. Immunocytochemistry for SCC tissues also detected SCC cells with high-expression of intracellular Vimentin at the adjacent cell layer of the stroma.

CONCLUSION : Stromal secretory factors may assist the EMT conversion in SCC cell.

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New role of PRIP in regulation of lipolysis

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We isolated PRIP as a novel molecule with similar domain organization to phospholipase C-δ1 (PLC-δ1). To explore physiological functions of the molecule, we generated PRIP knockout (PRIP-KO) mice and found that these mice exhibit hyperphagia, but the body weights changed low in comparison with those of wild-type (WT) animals; therefore we studied how PRIP regulates energy metabolism. The body fat content of PRIP-KO mice, quantified with computed tomography (CT) scan, was low. Moreover, oxygen consumption and carbon dioxide production rates, measured by a respirometer, were both up-regulated in PRIP-KO mice, and consequently, the body temperature was higher than WT control; suggesting PRIP-KO mice consume much more energy than WT mice.

To elucidate a molecular mechanism of energy metabolism via PRIP, we focused on lipolysis in white adipose tissue (WAT) and brawn adipose tissue (BAT) as well as thermogenesis in BAT. Lipolysis is a catabolic process leading to the breakdown of triacylglycerols stored in fat cells, and subsequent release of fatty acids and glycerol. This process is reversibly regulated by phosphorylation of perilipin and HSL. In this study, we elucidated that phosphorylation levels of these lypolysis marker proteins were higher than control, and free fatty acids level was also facilitated in WAT of PRIP-KO mice. We next clarified expression of uncoupling protein 1(UCP1) in BAT, which is thermogenesis marker protein, was increased in PRIP-KO mice, and this result was consistent with a higher body temperature of PRIP-KO mice. Taken together, these results demonstrated that PRIP is a new molecule for regulating the lipolysis and thermogenesis in adipose tissue.

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How many tooth-related genes are there in shark genome?

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BACKGROUND AND OBJECTIVE : There a variety of process in the evolution of teeth in animals. Human being exchanges their teeth only once in their life (diphyodont). On the other hands, sharks do it numerous times, that is called "polyphyodont". In order to know the mechanisms in which sharks can change teeth in numerous times, we first investigated how many tooth-related genes are present in the shark's genome, and attempted to compare human teeth-related genes with that of elephant shark.

METHODS : Human genes related with teeth are selected from the website "Gene Expression in Tooth". The selected genes were blast-searched to identify the orthologous genes in elephant shark from website "Elephant Shark Genome project", which has been published since 2007.

RESULTS : According to the information from the website of Gene Expression in Tooth published by Helsinki University, 314 genes related to growing and development of teeth in human were identified. Gene expression profile indicated that 103 tooth-related genes were expressed at Initial stage, 163 at Bud stage, 196 at Cap stage, 177 at Bell stage, 155 at Differentiation stage, 101 at Secretory stage, and 17 at Root development stage. Comparison of shark genome revealed that elephant shark carried approximately 41% of human tooth-related genes. Axin1, Pax9, Bmp4, fibroblast growth factor4, activin betaA, Msx1, Msx2, and fibronectin, which were the representative genes related to tooth-development, were present in elephant shark genome, however, amelogenin, enamelin, dentin sialoprotein, ameloblastin, fibroblast, osteocalcin, and osteopontin were not found.

CONCLUSION : It is suggested that sharks have unique mechanism to produce new teeth in their genome information.

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Evaluation of the course work education employing Cognitive Behavior Therapy

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OBJECTIVE : We have conducted course work (CW) in the Graduate Program for BioDental Education in Hiroshima University since 2008. CW was designed for graduate students to learn basic principles and techniques of biomedical research through practice. This year, we attempted to take up Cognitive Behavior Therapy (CBT) into CW to improve the students' positive attitude and quality of Education. Here, we show the results of the new trial and comparison with former CW will be discussed.

MATERIALS AND METHODS : Total 10 participants in 2010 and 9 participants in 2011 were enrolled in Startup-Program. The participants in 2010 evaluated themselves about the achievements of each topics item by item immediately after CW. The participants in 2011 evaluated themselves before and after CW. The evaluations were carried out using self-evaluation sheets. Then, we evaluated the students' positive attitude to the CW and alteration of motivation to the CW by grading into four ranks.

RESULTS : The participants in 2011 highly valued acquired skills and knowledge after the CW. They showed higher score in attitude to the CW than those participated in 2010.

DISCUSSION : The participants noticed what they should learn in the CW by adequately checking the aims of each topics before starting the CW. This trial indicated that it is important for students to clearly understand the lack of own knowledge before practice. Furthermore it is effective to improve their positive attitude and the will.

CONCLUSION : This trial indicated that it is effective to improve the way of education using CBT. We are also trying to provide a similar program for undergraduates. We suggest that our study will contribute to improve the quality of Education on our school's new CW programs.

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Complex formation mechanism of CDT holotoxin of *Aggregatibcter actinomycetemcomitans*

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Cytolethal distending toxin (CDT) is an exotoxin produced by several Gram-negative pathogenic bacteria and causes cell cycle arrest, cell distension, and apoptosis to various mammalian cells. CDT holotoxin is composed of 3 subunits, CdtA, CdtB, CdtC and complex formation is indispensable for the virulence of CDT. We have previously demonstrated that *Aggregatibacter actinomycetemcomitans* CdtA possesses a lipobox motif and is lipid-modified after/during secretion process. This CdtA undergoes posttranslational processing to become N-terminally truncated CdtA (CdtA'). However detailed biogenesis process of complex formation of CdtA (CdtA'), CdtB and CdtC remains ambiguous. During the study of complex formation, we found that membrane-bound CdtA rapidly undergoes processing and become CdtA' in the presence of CdtC, and complex of CdtA'/CdtC is recovered from supernatant fraction of centrifuged sample. This processing is inhibited by protease-inhibitor cocktail. The immediate processing of membrane-bound CdtA did not occur in the presence of CdtB. The CdtC-induced processing did not take place with free-form (unbound) CdtA. Processing of membrane-bound CdtA did not occur in the presence of CdtC-pretreated *E. coli* membrane suggesting that direct association of membrane-bound CdtA and CdtC is necessary for the processing. This association may activate membrane-bound protease (s) or make CdtA susceptible to membrane-bound protease (s). Subsequently, released CdtA'/CdtC complex may bind with soluble CdtB to form holotoxin in the periplasm.

In conclusion, our study suggests that membrane anchoring of CdtA and CdtA-CdtC assembly on membrane are important for the efficient formation of CDT holotoxin.

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MEPE-associated phosphorylated ASARM is a potential target to facilitate bone mineralization

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Members of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family, including matrix extracellular phosphoglycoprotein (MEPE), are non-collagenous extracellular matrix proteins and play roles in bone and tooth formation. A proteolytic fragment containing an acidic serine- and aspartate-rich motif (ASARM) of SIBLINGs inhibits hydroxyapatite crystal growth in vitro, suggesting that accumulation of ASARM in bone/tooth may cause mineralization defects. ASARM peptide is also a candidate substrate for the membrane protein PHEX (phosphate regulating endopeptidase homolog, X-linked) of which mutations cause bone and dentin mineralization defects. We found that Phex is a downstream target of fibroblast growth factor (FGF)23, a phosphaturic hormone secreted from osteoblasts/osteocytes and that MEPE-associated ASARM (MEPE-ASARM) peptide selectively inhibited matrix mineralization in multiple bone formation processes in vitro. In 18 amino acid residues of MEPE-ASARM, the possible PHEX cleavage and casein kinase II-phosphorylation ($Ser^{418/420/422}$) sites were indispensable for this effect. We therefore developed an antibody against human phosphorylated MEPE-ASARM ((p)ASARM). This antibody recognized (p)ASARM and MEPE, but not partially phosphorylated and non-phosphorylated ASARM which failed to inhibit bone mineralization. Anti-(p)ASARM antibody had neutralizing activity to the hypomineralization induced by (p)ASARM and other anti-mineralization agents. An excess amount of non-phosphorylated ASARM shifted the effective concentrations of (p)ASARM from higher to lower, while soluble PHEX abrogated the hypomineralizaiton effect of the phosphorylated peptide. Using immunohistochemistry, we found that (p)ASARM was primarily localized in osteocytes, osteoblasts, and bone matrices of mice, and staining intensity was extremely higher in mice deficient in Klotho, a coreceptor for FGF23 (*kl/kl* mice), versus wild type mice. Immunoprecipitation studies provided evidence for circulating (p)ASARM in mice and its increased levels in *kl/kl* mice. These results suggest that (p)ASARM and its specific antibody would be expected to be a biomarker of bone mineralization and an anabolic agent for bone formation, respectively.

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