2nd Hiroshima Conference on Education and Science in Dentistry

Hiroshima University Faculty of Dentistry

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Proceedings of 2nd Hiroshima Conference on Education and Science in Dentistry
October 6-7, 2007, in Hiroshima, Japan

Hiroshima University Faculty of Dentistry

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Printed in Japan

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The Role of Hiroshima University in Asian Development

T. Asahara

Hiroshima University, President

Hiroshima University was founded over 58 years ago and, with the notion of "a single unified university, free and pursuing peace," the university has steadily grown to become one of Japan's leading universities. Our university will continue to fulfill its universal mission as an institute of higher education, which is to "to cultivate outstanding human resources who can contribute to society and to undertake research that contributes to the development of humanity."

Furthermore, Hiroshima University seeks to live up to the role expected of universities in handling 21st Century global issues such as the population problem, environmental issues, world hunger issues, the energy crisis, the issue of peace, the threat of epidemics, and so forth. With the completion of the human genome in the late 20th Century and other rapid advances in scientific research, and the changes in the structure of Japanese society due to the falling birthrate and an aging population, human society is progressing speeds beyond our imagination.

In order for Hiroshima University to continue to develop in such a world, we believe that it is extremely important that we raise our international competitive power and make efforts which will define both HU's personality and distinctive features in the intensified global competition to come in all fields of study.

With "peace", "environment", and "medical care" being the keywords, I would like to introduce projects that highlight Hiroshima University's personality and distinctive features.

Firstly, with "the pursuit of peace" stated in the first of our Five Guiding Principles, and as a university located in the first city to experience the atomic bomb, Hiroshima, HU was the first university in Japan to establish the Institute for Peace Science as a academic research institute for peace science in 1975 to address the issue of international peace. Furthermore, in 1994, the Graduate School for International Development and Cooperation was established to promote research in international peace and work toward training human resources with the ability to contribute to the field of international peace and cooperation. We have included peace studies in other curriculums at the undergraduate level as well, so that we might cultivate human resources with a deep understanding of peace. In addition, we have begun the "Pilot Programme for Human Resource Development in Asia for Peacebuilding", a project from the Ministry of Foreign Affairs, which will train 30 civilians from Japan and Asia to become peacebuilders. It is our belief that from here forward, Hiroshima University will make a place for itself as a university with "liberal arts education" as a distinctive characteristic of its education and will contribute to Asian and world peace by working toward human resource development for peacebuilding.

Secondly, regarding 21st Century global environmental issues, research is progressing at our Graduate School of Engineering, Graduate School of Biosphere Science, and Graduate School of Science in environmental pollution, food and resource issues, the energy crisis, and others. In August of 2007, HU signed a comprehensive agreement for biomass research, which includes research and development, as well as human resources training in the fields of renewable energy and environmental society building with the use of biomass as a standard, with the National Institute of Advanced Industrial Science and Technology and it is our aim to become a biomass research hub in Asia in the near future. We have also had results with our research projects using jellyfish to evaluate the pollution and environment of the ocean. In order to prevent the environmental destruction, such as global warming, air, water, and chemical pollution, as well as atomic disasters, which threatens humanity's future, we believe that it is necessary from here forward to actively tackle these issues through scientific research and human resource development.

Thirdly, in the field of medical treatments, HU accepts young researchers from China and other Asian countries to study in a wide range of fields from fundamental research to clinical studies. HU is working towards training Asian medical personnel, and it also pursuing the development of medical science and medical treatment through joint research projects. In particular, at our Research Institute for Radiation Biology and Medicine, and on top of our large collection of documents on epidemiology, the likes of which the world has never seen, we're strongly engaged in radiation casualty research centered on low dose radiation disasters and genome damage research, which has accumulated excellent results internationally. For the effective and practical use of atomic energy, an important energy source in the 21st Century, it is crucial to perfect our preparations for radiation disasters. For this purpose, the role of Hiroshima University is a crucial one, and we plan to advance our research in radiation disasters even more in the future.

Finally, creating international networks is becoming gravely important as global competition grows

fiercer in every field, and the demand for international competitiveness increases. Currently at Hiroshima University there are a total of 800 international students enrolled in both our undergraduate and graduate programs, and 87% of those students are from Asia. From here forward, HU will promote international personnel exchanges and joint research centered around the countries of Asia, advance scientific research which can contribute to the future of humanity within the framework of an Asian network, and plans to work towards cultivating human resources with the ability to contribute to "building a society with a future full of hope."

Special Lecture

Special Lecture I

Salivary Diagnostics: The Scientific Foundations

University of California Los Angeles, Professor

David T. Wong

Special Lecture II

Oral Health Care Needs and Dental Education

Medical Education division, Higher education bureau Ministry of Education, Culture, Sports, Science and Technology, *Director*

Koji Miura, MD, MPH, PhD

Salivary Diagnostics: The Scientific Foundations

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ABSTRACT

The ability to monitor health status, disease onset and progression, and treatment outcome through non-invasive means is a most desirable goal in the health care promotion and delivery. There are three prerequisites to materialize this goal: specific biomarkers associated with a health or disease state; a non-invasive approach to detect and monitor the biomarkers; and the technologies to discriminate the biomarkers. A national initiative catalyzed by the National Institute of Dental & Craniofacial Research (NIDCR) has created a roadmap to achieve these goals through the use of oral fluids as the diagnostic medium to scrutinize the health and/or disease status of individuals. This is an ideal opportunity to bridge state of the art saliva-based biosensors, optimized to disease discriminatory salivary biomarkers, for diagnostic applications. Oral fluid being the "mirror of body" is a perfect medium to be explored for health and disease surveillance. The translational applications and opportunities are enormous. This review presents the translational value of saliva as a diagnostic fluid as well as the scientific rationale and foundation as a credible clinical diagnostics fluid.

Key words: Oral fluid, Saliva, Oral cancer, Nanotechnology, Proteomics, Genomics

INTRODUCTION

High impact human diseases, including cancer, cardiovascular, metabolic and neurological diseases, are challenging to diagnose without supplementing clinical evaluation with laboratory testing. Even with laboratory tools, definitive diagnosis often remains elusive. Three roadblocks hold back the realization of clinical diagnostics' potential - 1) definitive disease-associated protein and genetic markers; 2) easy and inexpensive sampling methods that effect minimal subject discomfort; and 3) an accurate, portable, and easy-to-use diagnostic platform. Saliva, a biofluid that is totally non-invasive and readily available, has long been recognized to address the second road block (Mandel, 1993). With the visionary investment by the National Institute of Dental & Craniofacial Research (NIDCR), saliva biomarker discovery and salivary diagnostic technologies are currently in development that are addressing the first and third roadblocks. It is safe to predict that the use of saliva for disease diagnostics and normal health surveillance is about five years away. This is an exciting time as we are seeing the applications of saliva diagnostics for oral diseases, which will soon to be followed by systemic diseases. This will truly allow the bridging of oral health research into systemic diseases via the biofluid the filters and processes itself from the vasculature that nourishes the salivary glands into the oral cavity (Figures 1 and 2). Oral fluid being the "mirror of body" is a perfect medium to be explored for health and disease surveillance. The translational applications and opportunities are enormous.

A growing number of proof-of-principle examples have been established for using saliva to monitor systemic diseases and conditions. The barriers to widespread implementation of saliva diagnostics derive from technological problems such as sensitivity, miniaturization, high throughput, automation, portability, low cost, high functionality, and speed to enable detection and measurements of multiple disease markers in saliva have largely been overcome. Techniques are emerging from a combination of miniaturization technologies and discoveries in many different fields of

biology, chemistry, physics and engineering are leading to high throughput, automated, portable, low cost, more efficient, and rapid biochemical analyses. Miniaturized diagnostic technologies will be able, with minute amounts of body fluids, to yield critical patient information reflecting health and disease status. These "lab-on-a-chip" platforms will be able to perform multiple operations in parallel in non-laboratory settings such as the field, factory, hospital clinic or home. It is envisioned that such technologies will allow the simultaneous assessment of multiple conditions of health and disease and provide clinicians with prevention and therapeutic strategies to meet patient needs.

VISION AND CHALLENGES

The post-genomic era provides opportunities for high-throughput approaches to genomics and proteomics. The novel technologies of miniaturization coupled with the highly parallel detection create the possibility of radically new ways to detect and diagnose health and disease states in an individual, even in remote or impoverished settings. These discoveries and technological advances in conjunction with the ability for disease diagnostics in a non-invasive biofluid would offer a revolutionary change in medicine.

There is a great need for convenient and accurate point-of-care disease diagnostic tools in a non-invasive manner. This is of particular relevance in the developing world where many health risks and illnesses remain poorly defined and receive inappropriate treatment. In addition, little information about the burden of disease is available to guide population health decisions.

The vision and challenge of saliva diagnostics is to discover the diagnostic potential and optimize engineering technologies for this biofluid. Figure 3 is a venn diagram which illustrates that within the spectrum of total human health and disease states (top circle), it is envisioned that some of these states will reflect themselves diagnostically in saliva via either proteomic or genomic information (lower left circle). How much overlap will the subset be remained to be determined. The lower right circle illustrates the technology development platforms necessary to advance the point of care detection capability of saliva.

The challenge to making saliva diagnostic a clinical reality is to establish the scientific foundation and clinical validations necessary to position salivary diagnostics to be a novel, highly accurate and feasible technologies to achieve definitive point-of-care assessment of individuals' health and disease status. Inherent in this vision is to establish the science and diagnostic targets in saliva and the development of robust, simple-to-use biosensor technologies for reliable and valid clinical applications.

SALIVA AS A DIAGNOSTIC FLUID

Saliva is a mirror of the body. The ability to utilize saliva to monitor the health and disease state of an individual is a highly desirable goal for health promotion and health care research. However, saliva diagnostics is a later bloomer, as only recently has there been a growing appreciation of saliva as a mirror of the body which can reflect virtually the entire spectrum of normal and disease states (Mandel, 1993). These include tissue levels of natural substances and a large variety of molecules introduced for therapeutic, dependency or recreational purposes, emotional status, hormonal status, immunological status, neurological effects, and nutritional and metabolic influences. A major drawback to use saliva as a diagnostic fluid has been the notion that informative analytes are generally present in lower amounts in saliva than in serum (Miller, 1994). With new and very sensitive techniques, the lower level of analytes in saliva is no longer a limitation. Almost anything one can measure in blood, one can measure in saliva. Saliva has been reliably used to detect HIV 1 and 2, and viral hepatitis A, B and C. It can also be used to monitor a variety of drugs including marijuana, cocaine and alcohol (Mandel, 1993).

There are compelling reasons to use saliva as a diagnostic fluid to monitor health and diseases. It meets the demands for inexpensive, non-invasive and easy to use diagnostic methods. As a clinical tool, saliva has many advantages over serum. Saliva is easy to collect, store and ship and can be obtained at low cost in sufficient quantities for analysis. For patients, the non-invasive collecting techniques dramatically reduce anxiety and discomfort and simplify procurement of repeated samples for longitudinal monitoring over time. For professionals, saliva collection is safer than blood tests, which could expose health care providers to HIV or hepatitis virus. Saliva is also easier to handle for diagnostic procedures since it does not clot, lessening the manipulations required. Saliva-based diagnostics are therefore more accessible, accurate, less expensive and present less risk to the patient than current methodologies.

DEVELOPMENT OF TECHNOLOGIES FOR SALIVA-BASED DIAGNOSTICS

Five years ago, in 2002, the NIDCR initiated a concerted research effort in the area of saliva diagnostics and progress is currently being advanced towards technologically viable systems moving towards commercialization. NIDCR funded seven UO1 awards to develop microfluidics and microelectricomechanical systems (MEMS) for saliva diagnostics. MEMS are integrating systems consisting of mechanical elements, sensors, actuators, and electronics on a common silicon substrate developed through microfabrication technology. These systems use small sample and reagent volumes coupled with integrated detection methods to perform analysis. The seven NIDCR-supported U01 awards focused on the development of microfluidic and MEMS technologies for measuring DNA, gene transcripts (mRNA), proteins, electrolytes and small molecules in saliva as well as overall profile correlates of a particular disease state, such as cardiovascular disease (Herr et al., 2007; Yager et al., 2006).

However, it is clear that none of the new technologies will become a practical and clinical reality without strong partnerships with industry early in the development stage. The reasons include the many challenges that such technologies face reaching the stage of fabrication, integration of individual components, validation, regulatory approval and finally commercialization. This has sparked a new initiative for the "Development and Validation of Technologies for Saliva-Based Diagnostics" in order for the currently developed academic saliva diagnostic technologies to team up with industrial partners to further develop functional prototypes and test their robustness for clinical applications for saliva based detection of clinical diseases. The outcome of this initiative is the anticipated commercialization of saliva based diagnostic technologies optimized for the detection of highly sensitive and specific detection of salivary biomarkers for human diseases. Four of the initial seven groups were recently renewed for five years for the second round technology optimization and commercialization goal.

DIAGNOSTIC MOLECULAR TARGETS IN SALIVA: THE PROTEOME AND THE TRANSCRIPTOME

In fiscal year 2003, NIDCR funded three UO1 awards aiming to comprehensively identify and catalogue human salivary proteins from the three major salivary glands. It is envisioned that the Human Salivary Proteome (HSP) will be a resource to help elucidate disease pathogenesis and evaluate the influence of medications on the structure, composition and secretion of all salivary secretory constituents.

Multiplexed proteomics platforms are currently explored by the UCLA Saliva Proteome Consortium in order to collectively decipher the human salivary proteome (HSP). In general, a "Divide and Conquer" strategy is used to comprehensively probe saliva proteome primarily using bottom-up proteomics. The proteins from whole or ductal saliva (parotid and SM/SL) are initially fractionated with a variety of separation techniques including reversed-phase LC, strong cation exchange (SCX) LC, gel filtration LC, Zoom isoelectric focusing (Zoom IEF), and ultrafiltration,. Secondly, the collected protein fractions are digested with a proteolytic enzyme, e.g., trypsin, and then analyzed with 1-D or 2-D LC-MS/MS. Finally, the acquired MS data are processed and submitted for database searching using Mascot database searching engine. We are also comprehensively cataloguing saliva glycoproteins using LC-MS/MS and glycoprotein pull-down method based on hydrazide chemistry (Ramachandran et al., 2006).

The multiplexed proteomic platforms have clearly deepened the HSP analysis. In total, we have catalogued more than 1000 proteins in whole saliva and the analysis of parotid and SM/SL saliva is near completion. We have also developed a saliva proteome knowledge base (SPKB) to centralize the acquired proteomic data and annotate the identified saliva proteins. The SPKB is fully accessible to the public (www.hspp.ucla.edu) for query of the identified proteins, which are linked to public protein databases. Comparative analysis of HSP and human plasma proteome (HPP) suggests that extracellular proteins are predominant in HSP whereas the membrane proteins are predominant in HPP. HSP proteins have significant binding and structural molecular activities whereas the HPP proteins show significant activities of nucleotide/nucleic acid binding. In terms of "biological process", a significant percentage of serum proteins are involved in cell cycle or signal transduction whereas a significant percentage of saliva proteins are involved in physiological or response to stimulus processes.

Similar to plasma/serum, many proteins (e.g., mucins and amylases) in human saliva are glycosylated. We have recently profiled saliva glycoproteins using a glycoprotein pull-down strategy based on hydrazide chemistry. In this approach, glycoproteins were coupled onto a hydrazide resin, the proteins were then digested and formerly N-glycosylated peptides were selectively released with the enzyme PNGase F and analyzed by LC-MS/MS. Employing this method, coupled with in-solution isoelectric focusing separation as an additional means for pre-fractionation, we identified 84 formerly N-glycosylated peptides from 45 unique N-glycoproteins (Ramachandran et al., 2006).

Of interest and in a very serendipitous manner, our laboratory has recently made the discovery that discriminatory and diagnostic human mRNAs are present in saliva of normal and disease individuals. The salivary transcriptome presents an additional valuable resource, the second saliva-based diagnostic alphabet, for disease diagnostics. Our first report of the salivary transcriptome, demonstrating that the normal salivary transcriptome consists of ~3000 mRNAs (Li et al., 2004). Of particular value is that of the 3000 mRNAs, 180 are common between different normal subjects, constituting the normal salivary transcriptome core (NSTC). To demonstrate the diagnostic and translation potential of the salivary transcriptome, saliva from head & neck cancer patients were profiled and analyzed. Four genes from the NSTC (IL8, OAZ1, SAT and IL1B) were able to discriminate and predict if a saliva sample is from a cancer or normal subject with a sensitivity and specificity of 91% respectively (ROC = 0.95) (Figure 5). While head & neck cancer was used as the first proof-of-principle disease for salivary transcriptome diagnostics, data will soon be available for systemic diseases. These data, while early and exploratory, provide sufficient rationale and demonstrate the urgent need to fully explore "Salivary Transcriptome Diagnostics" for major human disease translational applications. Adding to this urgency is our recent finding that the serum transcriptome from the same patients we examined for their salivary transcriptome yield 4 RNA biomarkers that have a sensitivity and specificity of 91% and 71% respectively (ROC= 0.88), demonstrating clearly that for oral cancer detection, saliva transcriptome diagnostics has a slight edge over serum (Li et al., 2006).

There are advantages of utilizing transcriptome markers for diseases diagnostics. The marker discovery

process is high-throughput using genome-wide microarray platforms. While the human salivary proteome has just been completed, the normal salivary transcriptome has been completed 2.5 years ago (Li et al., 2004). As a biomarker, RNA is as robust, as informative as any other analyte. Thus salivary transcriptome offers the combined advantages of high-throughput marker discovery in a non-invasive biofluid with very high patient compliance. Highly diagnostic RNA signatures have been identified for head & neck cancer and two other major human systemic diseases. The value of salivary transcriptome research can also be gauged by the commercial section launching two products for salivary RNA stabilization and isolation (RNAprotectR Saliva from QIAGEN and Oragen-RNA from DNA Genotek).

Of interest is our recent study into the relationship of the salivary proteome and transcriptome. We have conducted the concurrent proteomic and transcriptomic profiling of whole saliva samples from three healthy subjects to test if there is co-existence of salivary proteins and their counterpart mRNAs in human saliva. Of the function-known proteins identified in WS, more than 60% were also found present as mRNA transcripts. For genes not detected at both protein and mRNA levels, further efforts were made to determine if the counterpart is present. Of 19 selected genes detected only at protein level, the mRNA of 13 (68%) genes was found in saliva by RT-PCR. This study indicates that saliva transcriptome may provide preliminary insights into the boundary of saliva proteome (Hu et al., 2006b).

THE UCLA COLLABORATIVE ORAL FLUID DIAGNOSTIC RESEARCH CENTER

The UCLA School of Dentistry is engaged in both the technology development and the salivary proteome initiatives for saliva diagnostics. During the past four years, we have established the "UCLA Collaborative Oral Fluid Diagnostic Research Center" to develop the platform of using nano/micro technology to detect salivary protein and genomic biomarkers for point of care applications of high impact human diseases.

For salivary diagnostic technology development, we have partnered with engineers at the UCLA School of Engineering who are pioneers in the development of micro- and nano-electrical-mechanical systems (MEMS & NEMS) biosensors that exhibit exquisite sensitivity and specificity for analyte detection, down to single molecule level (Huang et al., 2002; Soong et al., 2000). Our research consortium has established a firm and committed collaboration toward the development of MEMS/NEMS biosensors for the real time, ultrasensitive and ultraspecific detection of salivary diagnostic analytes. This is a robust forum of interactions between engineers and biologists/clinicians towards the development of MEMS/NEMS biosensors for saliva-based disease diagnostics. Our prediction is that in ~2 years there will be "lab-on-a-chip" prototypes available for research as well as patient applications (St. John et al., 2004). The envisioned product is the "Oral Fluidic NanoSensor Test (OFNASET)". The OFNASET is a handheld, automated, easy to use integrated system that will enable simultaneous and rapid detection of multiple salivary protein and nucleic acid targets (Figure 4) (http://www.saliva.bme.ucla.edu/). This saliva biomarker detector can be used in dentist or health care provider's office for point of care disease screening and detection.

To fully utilize the diagnostic potential of saliva, one needs to comprehensively decipher and catalogue the informative components. Comparison of such a catalogue with a disease population will reveal diagnostic signatures that can discriminate normal and disease individuals. The salivary proteome presents one such resource. The UCLA group has already identified > 2,000 proteins in human saliva (www.hspp.ucla.edu) (Hu et al., 2005). We have begun to make translational discoveries into the salivary proteome for oral cancer (Hu et al., 2006c) and Sjogren's Syndrome patients (Hu et al., 2006a).

FUTURE PERSPECTIVES

While it is clear that there is a national agenda to turn saliva diagnostics into a clinical and commercial reality, much work needs to be done before this vision can be realized. There remains the need to identify definitive disease-associated salivary biomarkers (proteins and genetic) that can be use in conjunction with the technology platforms for saliva diagnostics. The scientific community is poised to develop and validate saliva based tests as a point of care chair side, portable and multiplexible devices to be used for diagnostic applications. Collectively, technology platform advancements and the identification and validation of robust and discriminatory suites of salivary biomarkers for disease diagnostics represent the necessary marriage to propel saliva diagnostics into a clinical and commercial reality (Figure 6).

FIGURE LEGENDS

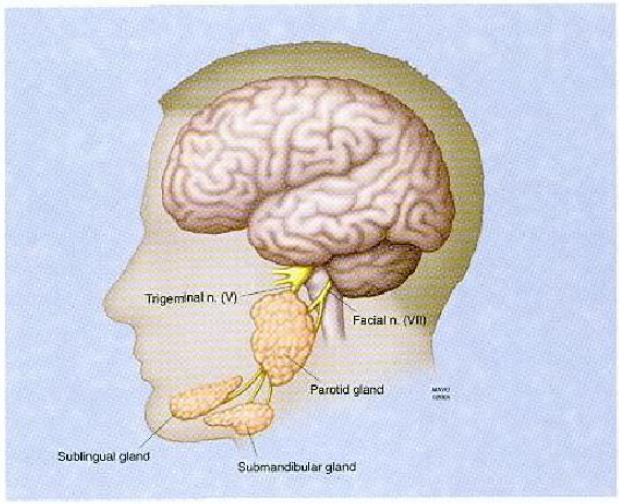

Figure 1:
Anatomical locations of the three major salivary glands: parotid, submandibular and sublingual.

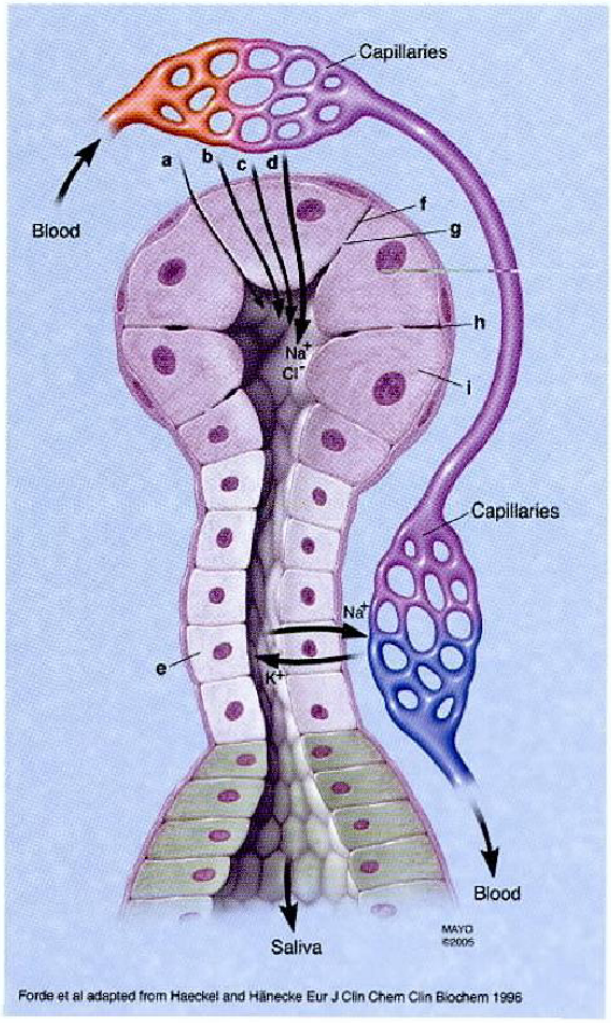

Figure 2:
Mechanisms of transport of proteins and ions from serum into salivary gland ducts.

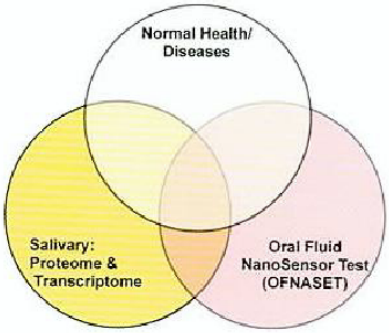

Figure 3:
Disease markers manifestation in saliva and their detection by saliva diagnostic biosensors (Oral Fluid NanoSensor Test, OFNASET).

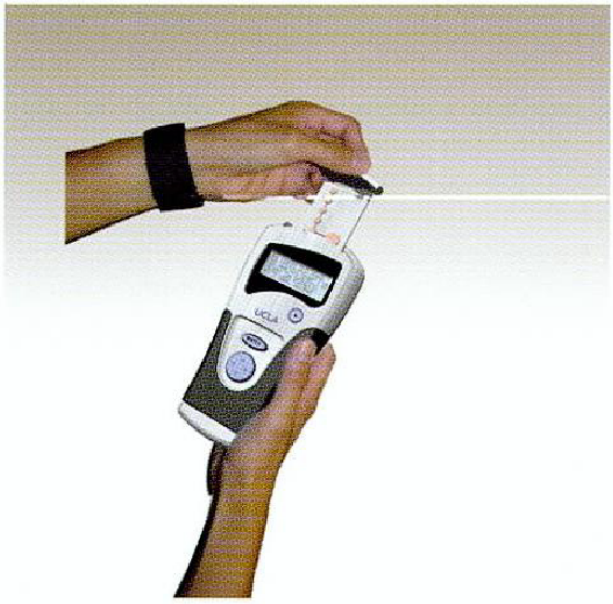

Figure 4:
UCLA's Oral Fluid NanoSensor Test (OFNASET).

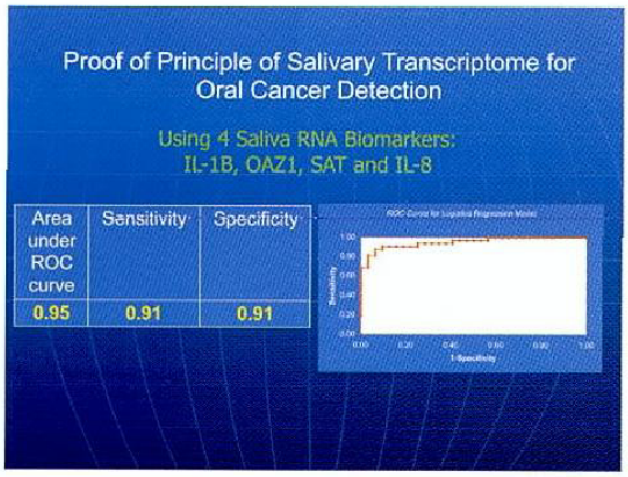

Figure 5:
Receiver operator characteristic (ROC) curve analysis for the predictive power of combined salivary mRNA biomarkers. The final logistic model included four salivary mRNA biomarkers, IL1B, OAZ1, SAT and IL-8. Using a cut-off probability of 50%, we obtained sensitivity of 91% and specificity of 91% by ROC. The calculated area under the ROC curve was 0.95

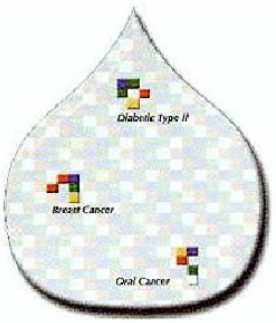

Figure 6:
A drop of saliva harbors a world of diagnostic information, proteomically and genomically. A handful of these analytes mark human diseases with great sensitivity and specificity.

ACKNOWLEDGEMENTS

Supported by PHS grants UO1 DE-15018, UO1 DE-16275, UO1 DE-17790, RO1 DE-15970 and RO1 DE-17593 and the Jonsson Comprehensive Cancer Center.

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Oral Health Care Needs and Dental Education

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1. Oral Health Status in Japan

The trends and the current status of dental diseases in Japan are mainly based on the results of the National Survey of Dental Diseases. This survey has been conducted every six years since 1957. The latest survey in 2005 indicated that tooth retention has improved and dental caries have declined. In contrast, periodontal diseases have not declined. The improvement of tooth retention might be due to by the increase of dentists. The major factor of caries reduction might be recent widespread use of fluoride toothpaste in Japan.

However, the importance of maintaining oral function of elderly person has been highlighted with the unprecedented rapid aging of the population.

Oral function is one of the major functions to sustain the life such as the prevention of aspiration pneumonia or the promotion of intake of nutrition. Moreover, from the view point of quality of life, it has significant role to enhance the happiness of enjoying taking meals.

Therefore, we should recognize the importance of oral function and the quality of dental care should be evaluated in this field.

2. Trends in Dental Utilization and Dental Practice in Japan

In Japan, all people receive health care under a public health insurance system. It is composed of several insurance schemes by different sectors of population and each person must subscribe to one of them. Regardless of insurance scheme, each patient sees any health care service providers at any time. The public insurance covers most of restorative, prosthodontic and surgical treatments except for personally preferred services, such as gold crowns, metal dentures, orthodontic treatments, and implants. (Orthodontic treatments for patients with craniofacial disorders are covered by the public health insurance or social welfare system.)

The "Patient Survey" from 1993 to 2002 revealed that the total dental visit rates for people aged 0-69 decreased, but increased among the elderly over age 70. Most dental visits were for treatments of dental caries or prosthodontics in all age groups. However, visits for dental caries or pulpitis decreased and those for periodontal diseases increased over these ten years.

3. Oral Health Care Needs in Japan

Since the prevalence of periodontal disease has not declined, prevention and treatment needs for periodontal disease is considered to increase in the future. Recent studies clarify the relationship between oral health and general health: for example, association between periodontal disease and diabetes, higher risk of cardio-vascular disease by periodontal disease, and higher risk of low birth weight infant by periodontal disease. Oral health care needs are thought to increase as the recognition of the importance of oral disease prevention and treatment becomes higher. In addition, since smoking is the major risk factor of periodontal disease and oral cancer, the importance of non-smoking support in dental field is expected to increase. Many youngsters start smoking in their teens, but they rarely visit medical institutions other than dental offices. Thereby it is difficult to support non-smoking in the early stage in which smoking habit is established. In the dental field, on the other hand, it is possible to support non-smoking in the early stage because many teenagers visit dental offices several times for the treatment of dental caries and gingivitis. Moreover, since there is an advantage that dental patients can easily recognize the adverse influence of smoking and effects of non-smoking due to the pigmentation of their teeth and gums, dental professionals should actively support non-smoking of teenage to the elderly patients. As already pointed out, maintenance of independence and improvement of QOL in the elderly people are a very important issue in Japan because of the rapid aging society. As recent studies report that maintenance and improvement of oral functions such as eating, talking and expressing have positive influence on ADL and QOL in the elderly: for example, prevention of pneumonia of the nursed elderly by professional oral care, improvement of ADL of the inpatient elderly in a rehabilitation hospital by dental treatments, and improvement of low nutrition in the nursed elderly by professional oral care, oral health care needs for the elderly who are independent, with risks of general disease and nursed have increased. It is considered that needs for not only outpatient but visiting dental treatments will increase.

4. Dental Education in Japan

There are 29 dental schools in Japan - 11 national, 1 prefectural (i.e. founded by a local government), and 17 private - representing approximately one school for

every 4.1 million people.

Undergraduate dental education requires six years, typically consisting of four years of preclinical education and then two years of clinical education.

High school graduates are eligible to enter dental school. Graduates are offered admission, but they account for fewer than 2% of the available positions.

Since 1990, Japanese dental education has undergone significant changes, with some dental schools implementing integrated curricula, problem-based learning tutorials, and clinical clerkships. A model core curriculum was proposed by the government in 2001 that outlined a core structure for undergraduate dental education. A nationwide common achievement test was instituted in 2005; students must pass this test to qualify for preclinical dental education. It is similar to the United States Dental Licensing Examination part 1, although the Japanese test is not a licensing examination.

The National Examination for Dentists implemented by Ministry of Health, Welfare and Labour is a 365-item examination that is administered once a year. In 2007, 3,200 applicants took the examination, and 2,375 of them (74.2%) passed. The Law of Dentist which was amended recently requires postgraduate training for one year after graduation from dental school. Residents are paid reasonably, and the work hours are limited to 40 hours a week. In 2006, a matching system was started; the match rate was 93.6% (87.6% for the university hospitals and 12.4% for other teaching hospitals and clinics).

Sustained and meaningful change in Japanese dental education is continuing.

5. Improvement of Dental Education

Dental education needs to respond to the disease structure change, aging society and oral health care needs change. According to the final report by the Expert Committee on Improvement of Medical Education presented on March 28, 2007, the following issues are suggested. Similar things can be applied to dental education.

① Improvement of entrance examination
② Improvement of dental education such as training of educators and researchers
③ Construction of persistent system of model core curriculum revision
④ Clinical clerkships
⑤ Improvement of new clinical medical internship in university hospitals
⑥ Specialist training
⑦ Promotion of clinical research
⑧ Organizational system playing an appropriate role in the university hospitals as educational and research hospitals
⑨ Environmental arrangement for increase of female doctors

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Contribution of Finite Element Modeling to Assessment of Mandibular Movements

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ABSTRACT

In clinical assessment of mandibular movements, the biomechanical models are powerful tools for establishing causal relationships and have led to updates of or new formulations on various insights into the function or dysfunction of the masticatory system. We have developed an individual three-dimensional modeling system for the temporomandibular joint (TMJ) based on the magnetic resonance (MR) images and the subsequent analysis of TMJ loading during jaw movements. This model analysis provided us available information about biomechanical environment within the TMJ in patients with temporomandibular disorders (TMDs). However, biomechanical models of the TMJ are obviously not perfect, while they are based on a number of assumptions and simplifications. Thus, the present study was designed to introduce previous biomechanical assessment system for jaw movement and the subsequent joint loading for each individual. Furthermore, we would like to present a challenging future planning for developing an integrated system of diagnosis and prognosis of treatment for patients with TMDs.

Key words: temporomandibular joint, mandibular movement, finite element analysis

INTRODUCTION

The human masticatory system consists of a mandible which is able to move in relationship to the skull base and is guided by two temporomandibular joints (TMJs) through contractions of the mastication muscles. To establish the contribution of each individual structure to jaw movements, one must explore the construction of the joints and the muscular system as well as the mechanical consequences of this construction. Insight into human masticatory system for each individual is of great importance to unravel the possible nature of temporomandibular disorders (TMDs) including masticatory muscle disorders.

In the field of clinical dentistry, TMDs are one of the major diseases as well as dental caries and periodontitis. TMDs have been defined as intraarticular morphologic abnormalities, such as different forms of disc displacement and degenerative joint disease. Epidemiologic surveys report that 20% to 25% of the population have symptoms of TMD (Carlsson, 1999), although only one fifth of those with TMD symptoms require treatment (Gray *et al.*, 1995). Internal derangement of TMJ (TMJ-ID) defined as an abnormal positional relationship of the disc relative to the mandibular condyle and the glenoid fossa, is accompanied with TMJ pain, clicking and/or crepitus, muscle tenderness, and limitation of mouth opening as the symptoms (Westesson *et al.*, 1986): these symptoms can reduce the QOL of the patients. Furthermore, the disc displacement preceded the onset of osteoarthritic changes in the TMJ (TMJ-OA) (Nickerson and Boering, 1989).

From a review of etiological events of TMJ disc displacement, trauma, functional overloading, joint laxity, degenerative joint disease and increased joint friction are considered to play a major role in the etiology of disc displacement (Nitzan, 2001). Therefore, evaluation of the biomechanical environment in the TMJ would lead to a better understanding of the inducing mechanism of TMJ pain and disability, which result in proper diagnosis and available treatment planning for TMDs.

However, there are several reasons why the

biomechanical environment in the TMJ is difficult to analyze. First, the masticatory system consists of a large number of muscles of various shapes and sizes, making it impossible to determine how they might cooperate to perform a certain task (Hannam and McMillan, 1994). Second, they have a complex architecture (van Eijden *et al.*, 1997), and their actions cannot be determined from their overall orientation only (van der Helm and Veenbaas, 1991). Third, the upper and lower jaws articulate through two very complexly shaped incongruent TMJs (Wish-Baratz *et al.*, 1996). Any simplification of these joints based on concepts usually used for other joints leads to considerable loss of functionality (van Loon *et al.*, 1999).

Recently, the application of biomechanical models has provided an adequate experimental framework to explore masticatory dynamics without several of drawbacks that accompany experiments with human subjects. They are powerful tools for establishing causal relationships in this field and have led to updates of or new formulations on various insights into the function or dysfunction of the masticatory system. During a decade, we have also developed an individual 3-dimensional modeling system for the TMJ based on the magnetic resonance (MR) image and the subsequent analysis of TMJ loading during jaw movements (Tanaka *et al.*, 2001). The present study was thus designed to introduce biomechanical assessment system for jaw movement and the subsequent joint loading for each individual. Furthermore, we would like to present a challenging future planning for developing an integrated system of diagnosis and prognosis of treatment for patients with TMDs.

IMPLICATION OF CLINICAL ASSESSMENT OF MANDIBULAR MOVEMENTS

Jaw movements are caused by the forces generated by the masticatory muscles. Furthermore, the joint reaction forces and occlusal forces are produced as the result of the jaw movements. The occlusal forces are essential for fatal masticatory function such as chewing, biting, and grinding. The joint reaction forces, if optimal, are of great importance for the development of the TMJ structure during adolescence and its maintenance in the adult (Copray *et al.*, 1985). Therefore, clinical assessments of stomatognathic function including TMJ function are necessary for dental practitioners.

In order to assess gnathostomatologic functions in patients, several analyses are conducted (Figure 1): measurements of maximum bite force, occlusal contact area, jaw movement, and muscle activities. Maximum bite force and occlusal contact area were measured by a Dental Prescale System (Fuji Film Co,Tokyo, Japan) consisting of pressure-sensitive sheets (Dental Prescale) and an analyzing computer (Occluzer; Fuji Film Co). The Dental Prescale responds to pressure by a color-developing chemical reaction. The amount of occlusal pressure and the size of the occlusal contact can be estimated by measuring the density and area data with a color image scanner contained in the Occluzer. The bite force can be calculated from the occlusal pressure and the size of the occlusal contact area. A pressure-sensitive sheet, Dental Prescale (50H, type R), was placed between the upper and lower dentitions, and the subjects were instructed to bite the sheet in the intercauspal position for 3 seconds.

Jaw movement and muscle activities were recorded simultaneously in all tasks. Activities of both masseter and anterior temporal muscles were recorded bilaterally, using bipolar surface electrodes, which are 6-mm diameter silver/silver chloride electrodes. For an optimal location of the electrodes, the maximum bulk of the muscle bellies was determined by palpation. The electrodes were placed in the direction of the muscle fibers with an interelectrode distance of 25 mm. The EMG signals were amplified and recorded with a sampling frequency of 1.5 kHz. The mean of rectified EMG data for every 0.03 seconds was stored in the data-recorder (RD-200T; TEAC, Tokyo, Japan). EMG was recorded during 3-second maximum voluntary clenching (MVC) with the teeth in the maximum intercuspal position. The subject was instructed to clench with maximum effort 3 times with an interval of 10 seconds. The integrated value of muscle activity was calculated during the median 2 seconds of the 3-second MVC and the values per 1 second were averaged. Furthermore, EMG was recorded during unilateral voluntary chewing of the 1.5-g gum. Each subject performed deliberate unilateral gum chewing for 50 seconds. The integrated values of masseter and anterior temporal muscle activity were calculated for every stroke, and the mean values were averaged. Jaw movement was analyzed using a 6-degree-offreedom optoelectric mandibular motion recording system (Gnathohexagraph, JM-1000; Ono Sokki, Yokohama, Japan), which consists of a head frame, a face-bow, light-emitting diodes (LEDs), CCD cameras, and a personal computer. A head frame with 3 LEDs was placed on the head parallel to the Frankfort plane of the subject, and the face-bow was set to the mandible through the use of a dental clutch. The dental clutch was attached to the labial surface of the lower anterior teeth by means of cryano-acrylate adhesive. Each subject was seated on a chair in an upright but relaxed position without a head support. Two CCD cameras were placed in front of the subject. The position of each LED was determined 3-dimensionally according to the parallax principle. The center point between the right and left lower central incisors was recorded by use of a pointer with 2 LEDs, and calculated based on the respective 3-dimensional positions of 6 LEDs attached to the head frame and face-bow recorded by the pointer. Movement of the incisal point was recorded during 50-second natural gum chewing. Referring to the muscle activity simultaneously recorded, the chewing side was determined for each stroke, and the numbers of strokes on the right and left sides were counted. Furthermore, all the chewing strokes on the working side during unilateral gum chewing were classified into several specific patterns: normal chewing pattern, crossover type, concave type, and reverse type. The latter 3 types are regarded as abnormal

chewing patterns. Condylar movements are also recorded. Sagittal condylar movement pattern (SCMP) can be categorized into six patterns: normal, figure-eight (early/intermediate/late), limited and other irregularities.

From these data obtained, dentists must determine a proper diagnosis for the patients and perform the treatment according to the assessment results. From these data, however, the biomechanical environment of the TMJ could not be analyzed sufficiently.

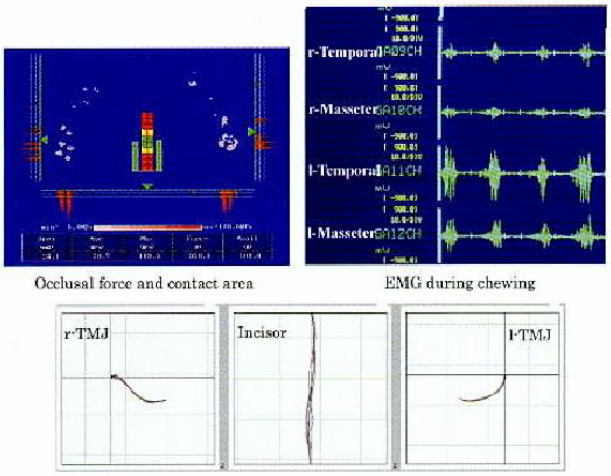

Figure 1:
Clinical assessments of mandibular movements

RECONSTRUCTION OF THE MANDIBLE INCLUDING THE TMJ

To evaluate the biomechanical environment of the TMJ, we have developed an individual 3-D modeling program for the TMJ based on MR images. For taking MR images, a 1.5-T magnet (Signa, General Electric) and a 3-inch dual-surface TMJ coil were used. The subject's head was placed in a head positioner with the Frankfort plane perpendicular to the scanning table, and the median sagittal plane of the head was kept approximately perpendicular to the scanning table. First, axial scans parallel to the Frankfort plane were taken in order to determine the long axis of the condyle. Based on the axial images, the sagittal plane of imaging was designed to be perpendicular to the long axis of the condyle. The coronal plane of imaging was determined to be perpendicular to the median sagittal plane and the Frankfort plane. The two sets of parallel lines in axial slice indicated the positions of the imaged sagittal and coronal planes. The sagittal images of the TMJ for each subject are acquired with the dentition in full intercuspal occlusion and maximum mouth opening. The coronal images were obtained and used for reconstruction of the medial and lateral portions. The scanning variables with a 3 inch dual-surface coil were 2200/20 milliseconds (repetition time TR / echo time TE) with the dentition in occlusion, and a 13 cm field of view. Contiguous 3 mm thick sagittal slices were obtained.

The reconstruction technique used in this study has already been described in detail elsewhere (Tanaka *et al.*, 2001). Briefly, from both the sagittal and coronal tracings the articular surfaces were approximated separately by using Coon's patches. Both approximations were fitted to each other and averaged. The shapes of the lateral and medial end portions of the condyle and articular disc could not be traced from the sagittal slices of the MR images (Figure 2). Therefore, the shapes of these areas were determined only by use of the coronal slices. The upper and lower boundaries of the articular disc were shaped according to the upper and lower articular surfaces. Interface elements were placed at the bone-disc crossing so as to allow the disc to deform and to move along the articulating surfaces without penetration. Because of the detective limit of the MR images, the tissue surrounding to the articular disc was modeled as a single connective tissue mass.

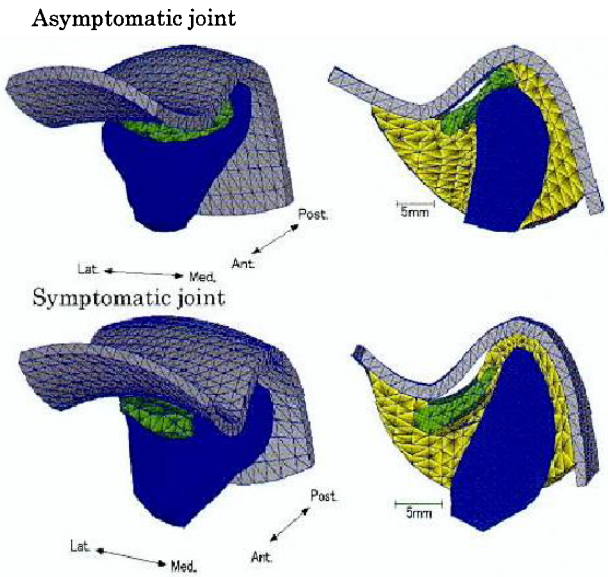

Figure 2:
Samples of 3-dimensional reconstructed TMJ models

STRESS ANALYSIS IN THE TMJ DURING JAW MOVEMENTS

The material properties of TMJ components used in this analysis were assumed to be linear viscoelastic and were taken from literatures. The model of the glenoid fossa was restrained for all degrees of freedom at its superior region. The mandible was modeled as a rigid body. It was constrained at the rearmost point of occlusal contact and at the central point of the anterior teeth on the mandible for mediolateral displacements, leaving three degrees of freedom in the sagittal plane. Contact in the TMJ was modeled by using contact elements between the disc and both articular surfaces with a frictional coefficient of μ = 0.01 for both TMJ models (Tanaka *et al.*, 2004; Kawai *et al.*, 2004). Joint loading was simulated with spar elements representing forces of 4 unilateral masticatory

muscles (masseter, temporalis, and medial and lateral pterygoid muscles). The insertion and origin points of these muscles, together with the central point of the anterior teeth and the rearmost point of occlusal contact, were estimated from the frontal and lateral cephalograms of each subject (Figure 3). The muscles were linked to the mandibular rigid body at their insertion points. For each muscle, the two points of its insertion and origin defined its line of action. The muscle forces were determined based on their physiological cross-sectional areas (PCS) and maximum muscle forces, and incorporated as spar elements. The stress analysis was executed on a personal computer with the finite element software, ANSYS from ANSYS Inc. (Houston, USA).

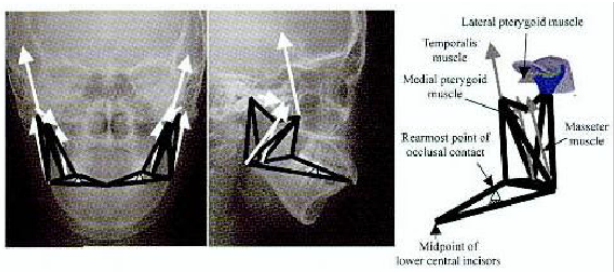

Figure 3:
Frontal and lateral cephalograms and the rigid link of th emandible with muscle spar elements and rearmost point of occlusal contact

Figure 4 shows a sample result of stress analysis in the TMJ disc during prolonged clenching (Hirose *et al.*, 2006; Tanaka *et al.*, 2007). For a healthy joint, the disc at the onset of clenching (time = 0 sec) appeared to be loaded in its lateral and central areas. The largest values of the stress (maximum: 0.91 MPa) were located in the central part of the intermediate zone and its lateral area. After 2 min, the stress in the disc leveled off. After 10 min of clenching, the largest stress appeared in the central part of the intermediate zone and the posterior band (maximum: 1.20 MPa). In the retrodiscal tissue at the onset of clenching, a relatively small stress (0.021 MPa) was found adjacent to the posterior band of the disc which decreased with time. After 1 min, the stress reached an almost steady level (maximum: 0.01 MPa), which implies that stress relaxation occurred in the retrodiscal tissue. Figure shows the displacements of five different points in the disc during clenching. All of them displaced anteriorly during the first 1-3 min of clenching. The anterior, central, lateral and medial disc points showed a 0.25-0.4 mm anterior movement after 10 min of clenching. The posterior point initially moved about 0.25 mm anteriorly and after 30 sec it moved posteriorly (about 0.07 mm). In the symptomatic joint a stress concentration was predicted in the medial and posterior part of the disc at the beginning of clenching. The maximum stresses (0.14 MPa) were located in the medial part. After 3 min, the stress in the disc reached almost steady level which was maintained during the rest of the clenching period. The retrodiscal tissue of this model exhibited larger stresses at the beginning of clenching compared to the asymptomatic one. The stress level kept about constant throughout the clenching period, which implies that in the retrodiscal tissue stress relaxation did not occur. The five reference points of the disc moved gradually in anterior direction throughout the 10 min of clenching. The amount of displacement was 0.40-0.44 mm after the clenching period, which was larger than in the asymptomatic joint.

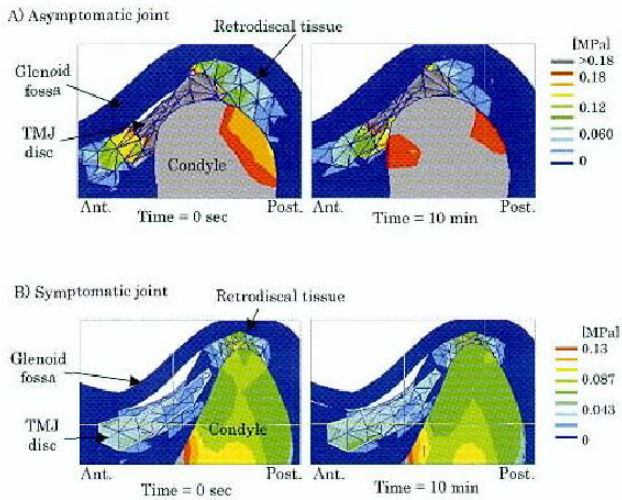

Figure 4:
Von Mises stress distributions in the TMJ disc and retrodiscal tissue of the asymptomatic (A) and symptomatic joints (B) during prolonged clenching

These results provide an important clinical indication such as the best way to reduce the damage due to parafunction (e.g., prolonged clenching and bruxism). Furthermore, the information about the biomechanical environment of the TMJ obtained this system enables us to do order-made treatment for each patient with TMDs.

LOOKING TO THE FUTURE

The finite element method has been proven to be a suitable tool for approximating the distribution of loads in the structures of the TMJ. Since 1990, several three-dimensional FE models of the TMJ including the disc have been developed (Beek *et al.*, 2000; Donzelli *et al.*, 2004; Koolstra and van Eijden, 2005; del Palomar and Doblare, 2006). Finite element analysis has been successfully used in this field because it enables us to estimate stresses in the TMJ without an invasive approach. These model analyses have provided information about biomechanical environment within the TMJ during mandibular movements. However, most of these models have been a standard model which was developed based on the human skull or a specific person. These enable to construct a precise geometrical model, but are not used for individual modeling of

living subjects. Therefore, we have been developed a 3-dimenisonal reconstruction system by which personal TMJ model is developed from individual MR images. However, we had to manually trace the contours of the joint components from MR images, discretize for each image, and pile up to represent the three-dimensional geometry of each TMJ component, and it takes a long time (1-2 weeks) for us to reconstruct one TMJ model by use of this system. This is because serial images obtained by MRI with a 1.5-T magnet were unclear so that we could not develop automatic 3-dimensional reconstruction system for TMJ modeling. Furthermore, the loading condition for stress analysis was likely to be determined from the EMG data recorded during jaw movements. However, there are several limitations to the collection of experimental data on the masticatory muscle activities. For instance, some of the masticatory muscles run deep and are partially hidden behind bony structures, which prevents easy access for EMG measurements (Murray *et al.*, 1999). Furthermore, many jaw movements are relatively small, posing stiff challenges to experimental systems designed to record relevant properties adequately (Naeije *et al.*, 1996). Considering resolution of these problems, recently we have attempted to make an integrated reconstruction system for the TMJ. High resolution MRI with a 3.0-T magnet (General Electric) and newly developed micro coil will enable us to reconstruct 3-dimenisonal finite element model of the TMJ automatically. Furthermore, by using a newly developed mandibular tracking device mandibular and condylar displacements during movements can be recorded in the same coordinate system of MR images. The condylar displacements recorded by the mandibular tracking device can be used as the loading condition for stress analysis. This enables us to avoid using incomplete EMG data as the loading condition.

CONCLUSIONS

Obviously, biomechanical model of the human TMJ and its application to stress analysis during mandibular movements are not perfect, while they are based on a number of assumptions and simplifications. In contrast, it is also true that better understanding of biomechanical environment within the TMJ is absolutely necessary for the diagnosis and prognosis of treatment of masticatory dysfunction. Future studies are required to measure and visualize the biomechanical environment within the TMJ during mandibular movements in clinical aspect.

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Newly Developed Device to Measure the Stability of Dental Implants

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ABSTRACT

Resonance frequency (RF) analysis technology was used to design a new dental implant stability detector. To calibrate and test the performance of this novel apparatus, the implant stability status was also detected clinically using our device at 2, 4, 8, and 12 weeks after surgery. In our clinical tests, an initial RF value above 10.0 kHz indicated that the implants were ready to accept functional loading, while values in the 4.0~10.0-kHz range reflected the need for further osseointegration. These results indicate that our new device may be useful in clinical settings for evaluating the healing status of recently placed implants.

Keywords: resonance frequency, dental implant, device

INTRODUCTION

Immediate loading refers to a restoration placed in occlusion with the opposing dentition within 48 hours of implant placement (Cochran *et al.*, 2004). When the appropriate implant conditions are present, most implants can be immediately loaded. Much of the research indicates that the success rate of immediate loading is good (Andersen *et al.*, 2002), but primary implant stability is one of the principal factors governing a dentist's decision concerning the ability of a given implant to accept immediate loading. However, few devices and methods are presently available for accurately detecting implant stability immediately after placement.

Results from several recent studies have demonstrated that the resonance frequency (RF) can be used to monitor the process of osseointegration after dental implant emplacement (Meredith, 1998). Osstell (Integration Diagnostics, Göteborgsvängen, Sweden), an RF device based on utilizing the harmonic response to monitor a dental implant's status, became commercially available in 2000. Recently, this device has been used in implant research to compare success rates of conventional and early-loading implants (Smet *et al.*, 2005), evaluate the survival rates for transmucosal implants immediately restored with single crowns (Cornelini *et al.*, 2004), monitor differences between immediate and standard delayed-loading implants (Bischof *et al.*, 2004), profile physiological and geometric factors affecting immediate-loading implants (Nedir *et al.*, 2004), and measure the stability achieved with one-stage surgical procedures (Monov *et al.*, 2005). However, a clinical investigation indicated that temporary replacement, removal, and sterilization of the transducer are time-consuming and less cost effective (Nedir *et al.*, 2004). In addition, the [please spell out]i s q (ISQ) level is often influenced by its orientation to the alveolar ridge due to Osstell's L-shaped transducer (Balshi *et al.*, 2005).

In this study, the capability of a novel experimental RF-detection device based on an impulse force triggered to monitor dental implant stability was tested. To reduce the time required for measurements, a minimum-contact device was designed that requires no additional installation and/or disassembly.

MATERIALS AND METHODS

Device development

As shown in figure 1a, our new device incorporates a minimum-contact transducer and an attached hand piece. This handle can be rotated to improve assess in the limited space of the oral cavity (Fig. 1a). The device consists of two sections of an electromagnetic coil that provide the driving feedback for a demagnetized-iron impact head (Fig. 1b). When an impulse current passes through the first coil section, the generated electromagnetic field attracts the impact head and drives it to strike against the healing abutment. The second section generates an electromagnetic field in the opposite direction, retracting the impact head to its original position. When the impact head strikes the test implant, the resultant vibration is detected via a piezoelectric microphone, and data are transferred to a spectrum analyzer (Implomates System, Biotech One, Taipei, Taiwan; resolution 50 Hz). The specific resonance frequency of the tested implant is determined by means of the relatively highest point of the peak value of the vibration amplitude.

Clinical data collection

Data for continuous RF measurements of 11 implants were collected from seven patients (six male and one female patient), all of whom (mean age 31.4 years, range 25-48 years at the time of surgery) were fully informed with respect to the study protocol before signing informed agreements. The recruitment criteria were no history of oral disease or dental implant

surgery. The edentulous areas were located at the mandibular premolar or first molar (Fig. 2A). The 3i implants (3i Innovation, city?, FL) were placed in the mandible according to the manufacturer's guidelines for the one-stage procedure (Fig. 2B). All implants were covered with a 4-mm healing abutment to avoid oral-fluid contamination. Resonance frequencies were measured using our newly designed apparatus immediately after the implants were in place (week 0) and at weeks 2, 4, 8, and 12 after implantation surgery (Fig. 2C). Detection was performed in the buccal-lingual direction. After a healing period of 12 weeks, the patients received their prostheses using the classical procedure.

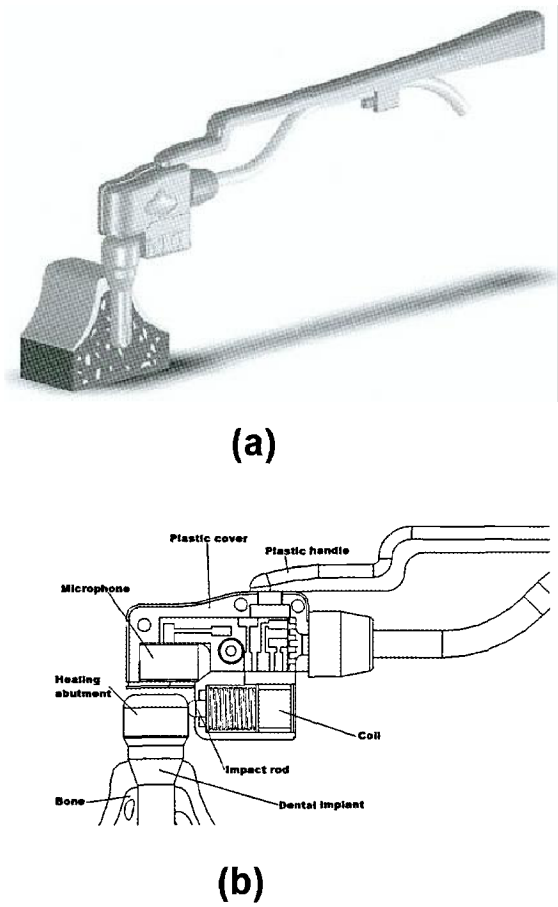

Figure 1.
Diagrams of the resonance frequency (RF) detector used in this study. (a) Designed as a minimum-contact probe, vibration of the test implant is triggered by an impact rod. (b) Sagittal section of the device showing the electromagnetic drive actuator and non-contact microphone.

RESULTS

In figure 3, the tested implants are divided into three groups. In group I, the initial RF values were above 9 kHz, remaining high and reaching a plateau of $\geq$ 11 kHz by week 12. In group II, the initial RF values of the implants were concentrated between 3.58 and 5.3 kHz. These implants remained in excellent condition throughout the course of the experiment and completed the osseointegration process. By week 12, the RFs for all group II implants were > 9.8 kHz. In group III, one implant with an initial RF value of 3.56 kHz did not demonstrate an increasing trend in RF values after the first 2 weeks of healing. The osseointegration of the implant failed, and by week 12 it had loosened, with a final RF value of 3.85 kHz. Our short-term results demonstrated a 91% success rate during the first 12 weeks.

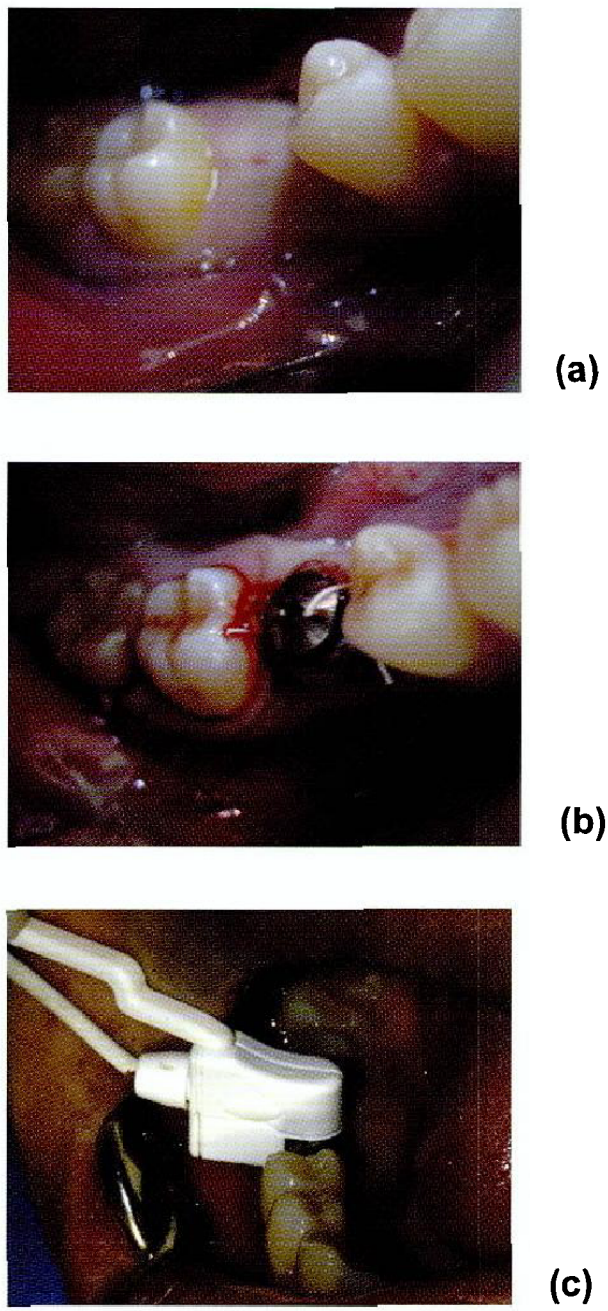

Figure 2.
Entire surgical and test procedure: (a) location of an edentulous area; (b) general procedure for implant placement; (c) resonance frequencies of the test implants detected using the new device.

To assess the relationship of RF values between immediate- and delayed-loading implants in this study, the resonance frequency increase ratio (RFIR) was defined as the ratio between the initial RF and the analogous value in week 4. The mean RFIR for group II implants (1.98 ± 0.31) was significantly higher than that of group I (1.09 ± 0.11; $p < 0.005$). The plot in figure 4 reveals the linear relationship between the RFIR of each test implant and its initial RF (y = -0.126x + 2.50, $R^2 = 0.811$, $p < 0.05$).

DISCUSSION

Osstell was the first commercially available RF device for testing dental implant stability (Barewell *et al.*, 2003). However, it is somewhat inconvenient and time consuming to use (Nediret *et al.*, 2004), and it may affect the implant/bone interface in the early healing stage. The Osstell transducer must be screwed into the test implant with a torque of 10 N-cm, almost half the force used to place an implant. Thus, the mechanical effect of disassembling the healing abutment on the interface in the early stages of osseointegration should be considered. Therefore, our novel transducer was designed as a minimum-contact device, with no torque force required during transducer application.

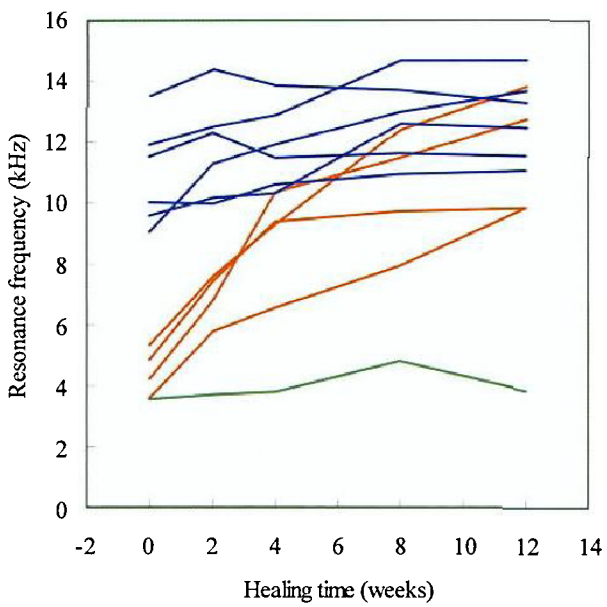

Figure 3.
Resonance frequency (RF) healing curves from test implants in patients during 12 weeks post implantation. Solid, and short- and long-dashed lines denote groups I, II, and III (with initial RF values of > 9, 3.5~6, and < 4 kHz), respectively.

It has been reported that primary stability can serve as a useful predictor of osseointegration (Bischof *et al.*, 2004). Further, it has been demonstrated that good primary implant stability, as measured by the RFA, does not significantly increase during the osseointegration period (Bischof *et al.*, 2004). We found that implants with higher stability indeed had relatively stable RF values during the entire healing period with a mean RFIR of 1.09 ± 0.11 (Figs. 3, 4). As constant or slightly increasing RF values have been reported during the first 4~6 weeks for immediate-loaded implants (Bischof *et al.*, 2004), it appears reasonable to suggest that when implants have initial RF values above 10 kHz (as measured by our device) the implant is ready for immediate loading.

A number of researchers have proposed that when the initial ISQ is above 60, an implant is suitable for immediate loading (Bischof *et al.*, 2004; Nedir *et al.*, 2004) while values below 40 may serve as a warning of early failure (Glauser *et al.*, 2004). Interestingly, this proposed functional threshold of an ISQ of 60 is equivalent to an RF of 6.10 kHz (Glauser *et al.*, 2004), much lower than our experimentally determined limit of 10 kHz. A negative relationship has been reported between RF values and the effective vibration length above the bone level (Bischof *et al.*, 2004; Huang *et al.*, 2003). Since the Osstell transducer is an L-shaped device that must be attached to the top of the tested implant, the effective vibration length of the test implant is greater than that of our device.

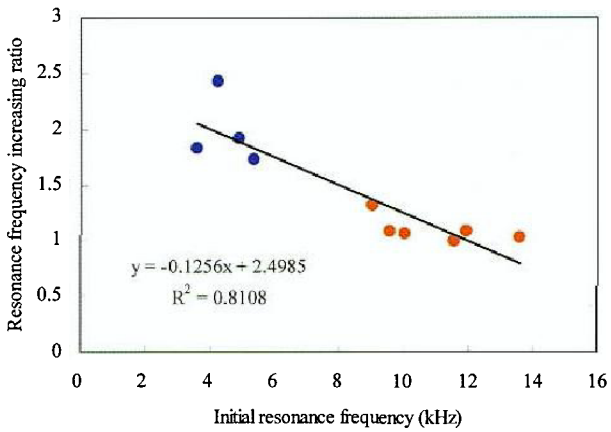

Figure 4.
Relationship between initial resonance frequency (RF) values and RF increase ratios of successful implants at 4 weeks after surgery. Open and closed circles denote group I and II implants, respectively.

The initial RF values for our tested implants varied (Fig. 3), probably reflecting the marked inter-individual variations in distributions of cortical and trabecular bone, as well as differences in various locations [of what?] within the bone. After analyzing the RFA data for 120 one-stage implants, Zix et al. (2005) suggested that a single RFA measurement for an implant at a given time-point cannot fully predict its future performance. Repeated measurements of an implant over a period provides a more-credible prognosis of its future status. Glauser *et al.* (2004) suggested that low RFA levels after 1 and 2 months provide an indicator of risk for future failure. Although implants with a lower initial RF revealed significantly higher

RFIR values (Fig. 4), the RFIR was defined in this study for early diagnosis but not for judging the time-point of functional loading. However, it appears reasonable to suggest that the time for functional loading of these implants is when their RF value plateaus or exceeds 9 kHz. Nedir *et al.* (2004) also reported that implants with lower initial ISQ values demonstrated larger ISQ increases during the healing period. In contrast, higher initial stability is associated with a more-stable ISQ value. In addition, only implants with the highest stability reveal stability decreases during the first 4 weeks before becoming stable. These above results are substantially in accord with our own findings.

Based on these results, therefore, it appears reasonable to suggest that our novel RF device may be suitable for evaluating the integrity of bone union during the osseointegration process while providing functional superiority without impacting the restoration process. The greatest advantage of this new device is its ergonomic superiority. Thus, we propose that the novel RFA apparatus designed for and used in this study may afford substantial benefits in future advanced experiments, as well as offering significant promise for future clinical use.

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Application of Rapid Prototyping in Dentistry

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ABSTRACT

Rapid prototyping is the automatic fabrication of physical objects by joining material (e.g., liquid, powder, or sheets) together layer by layer. The rapid prototyping can fabricate geometrically-complex objects even though they can not be fabricated by other conventional fabrication methods. The rapid prototyping is not suited to mass production of single item, but the rapid prototyping is convenient to single piece production of custom-made products. Since this characteristic suits individualized (so-called tailor-made) medical services, the rapid prototyping has been applied to medicine and dentistry. In this paper, the authors outline the following types of the rapid prototyping: stereo-lithography; fused deposition modeling; 3D printing; and selective laser sintering. Then the authors survey various applications of the rapid prototyping in dentistry, which include: fabrication of dental and facial prostheses; oral and maxillofacial surgery; and tissue engineering. Different types of rapid prototyping technologies are applied, depending on the purposes of applications.

Key words: Rapid Prototyping, Dental and Maxillofacial Prostheses, Oral and Maxillofacial Surgery, Tissue Engineering, 3D Model

INTRODUCTION

Rapid prototyping (RP) is the automatic fabrication of physical objects by joining material (e.g., liquid, powder, or sheets) together layer by layer. RP can fabricate geometrically-complex objects even though they can not be fabricated by other conventional fabrication methods.

The first techniques for RP were emerged in the 1980s, which aimed for fabricating prototypes rapidly. In addition to prototypes, the techniques are recently used to fabricate final products or their components with high quality. Therefore the term, "solid freeform fabrication," is sometimes used instead of "rapid prototyping."

RP is not suited to mass production of single item, but RP is convenient to single piece production of custom-made products. Since this characteristic suits individualized (so-called tailor-made) medical services, RP has been applied to medicine and dentistry. In this paper, we outline the types of RP and survey the state-of-the-art applications of RP in dentistry.

TYPES OF RAPID PROTOTYPING

As shown in Fig. 1, RP takes a three dimensional model (3D model) as input, which is virtual on a computer and which is usually made by a Computer-Aided Design (CAD) system or other software handling 3D models. In the medical or dental fields, 3D models are made from: the image data of Computed Tomography (CT) or Magnetic Resonance (MR); or the data scanned by a 3D scanner which measures 3D form of an object. Next RP divides the 3D model into horizontal cross sections on the computer, and then RP fabricates a physical thin layer corresponding to each cross section, one after the next until the whole physical object is completed. Though there are various types of RP, in this section we describe several types that are well-known and well-used in dentistry.

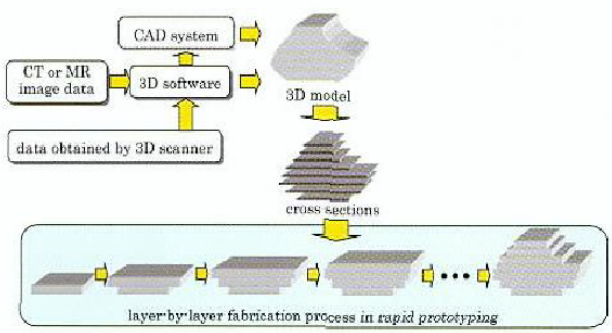


Figure 1:
Fabrication of a physical object from a 3D model by rapid prototyping

Stereo-lithography

Stereo-lithography (SLA) fabricates a physical object by curing photosensitive resin using ultraviolet (UV) laser irradiation. As shown in Fig. 2, SLA emits laser beam on the surface of liquid resin so that the resin cures. After one layer of resin exposed to UV radiation cures, a table moves down by a single layer thickness in the bath of liquid resin. The surface of the previous layer is coated with a new layer of liquid resin which is subsequently cured and is bonded to the previous layer. This process is repeated until the

model is completed. The structure for supporting the cured resin is also created during the process mentioned above if the cured resin may droop down. Figure 3 shows an example of jaw model with the support structure. Such a support structure must be removed after the model is completed.

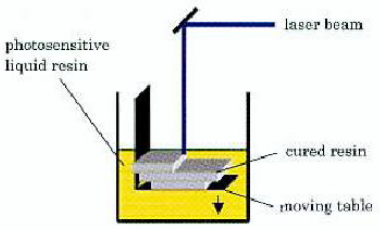


Figure 2:
Principle of stereo-lithography

Figure 3:
An example of jaw model with support structure

Fused Deposition Modeling

As shown in Fig.4, Fused Deposition Modeling (FDM) extrudes fused thermoplastic material from a nozzle, and the extruded material is deposited on a table to make one layer of a model. Once one layer has been made, the nozzle moves up slightly and the next layer is made on the previous layer in the same way. This process is repeated until the model is completed. FDM creates the structure for supporting the model, similarly to SLA. However the support structure is made of a different material which is extruded from another nozzle and which can be removed easily after the model is completed. Though Acrylonitrile Butadiene Styrene (ABS) is usually used as the thermoplastic material, various types of materials can be used for fabricating the models.

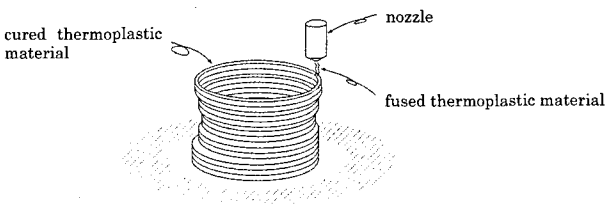


Figure 4:
Principle of fused deposition modeling (Furukawa et al., 2002)

3D printing

3D printing technologies are classified into two types: 3D printing using powder; and 3D printing without using powder.

3D printing using powder selectively bonds the powder of material (e.g., resin, plaster, or starchy powder) by jetting a water-based adhesive on the powder. An inkjet print-head is used to jet the adhesive, as shown in Fig. 5. This is the reason why this technology is called 3D printing. Once one layer has been made by bonding the powder, a table moves down slightly and the previous layer is covered with new powder to make the next layer. This process is repeated until the model is completed. 3D printing does not have to create the support structure since left powder, which has not been bonded, can support the model. In general 3D printing using powder excels other methods in time consumption, cost, and easiness of operation. Moreover 3D printing has the unique advantage of being able to make color models.

The other types of 3D printing do not use the powder. One of the types jets wax instead of the adhesive from an inkjet print-head and deposits the wax on a table. Another type jets photosensitive liquid resin on a table from an inkjet print-head. The jetted liquid resin on the table is cured immediately by UV lamp mounted in the print head. Figure 6 shows the equipment of this type, EDEN (OBJET), which we use.

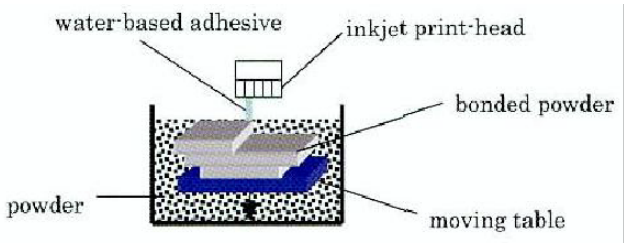


Figure 5:
Principle of 3D printing using powder

Figure 6:
EDEN(OBJET): 3D printing using photosensitive resin

Selective Laser Sintering

Selective Laser Sintering (SLS) selectively fuses the powder of material (e.g., thermoplastic, metal, or ceramic powder) by emitting a high power laser (e.g., CO2 laser) on the powder. Once a layer has been made, a table moves down slightly and the previous layer is covered with new powder, similarly to 3D printing using the powder. SLS does not require the support structure since the left powder can support the model. SLS is the only RP technology that can deal with metals.

APPLICATION TO PROSTHESIS FABRICATION

Dental Prostheses

Crowns and Bridges

The first application of RP to dental prosthesis fabrication was achieved by the 3D printing using wax (wax plotting). WaxPro (Cynovad) fabricates the wax patterns of copings, crowns and bridges by the wax-plotting method, after the design of them by Pro50 (Cynovad). Since WaxPro can fabricate a lot of wax patterns at a time, this system can reduce the time for waxing-up. Neo (Cynovad), which is a dental CAD/-CAM system, can design and create the wax patterns of all types of restorations. WaxPro can fabricate up to 150 wax copings per 8 hours and Neo can fabricate up to 200 units per day. In addition, these systems can achieve high precision fit and consistent wall thickness, not depending on individual know-how and skill. The stereo-lithography can also fabricate the master models of all types of restorations in the similar way (Witkowski, 2005). Though the models are not made from wax but acrylic resin, the models can be used for casting.

The application of SLS to dental prosthesis fabrication (Ueda, 2003; Witkowski, 2005) is one of the notable approaches in dental CAD/CAM area. In this application, the powder of titanium or other metals is used for fabricating crowns and bridges.

It is required to make a comparison in time consumption and cost including equipment investment cost between the RP technologies mentioned above and removal fabrication method that is usually used in dental CAD/CAM systems.

Dentures

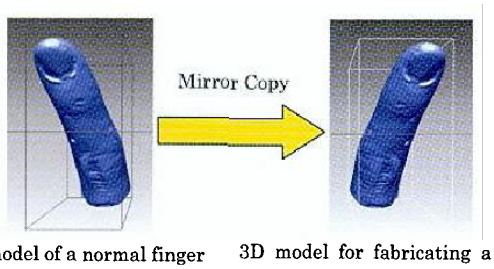
The 3D printing using wax is used for fabricating trial complete dentures (Nakanoda et al., 2007). In this approach, artificial teeth and edentulous model are scanned by a 3D scanner and the scanned data are used for the artificial teeth arrangement and gum forming on a computer. Then trial complete denture is made by the 3D printing, according to the design on the computer. SLA has also applied to fabricating complete dentures (Maeda, et al., 1994).

RP is also applied in removal partial dentures (Bibb et al., 2006). In this application, a cast of a patient is scanned, and the scanned data are used for the dental surveying and pattern building on a computer. A sacrificial pattern is fabricated by RP. Using the pattern, a framework is produced through casting. The framework is successfully trial-fitted to the patient.

Facial Prostheses and Others

There are several applications of RP to facial prosthesis fabrication. Since RP can not deal with silicone, which is usually used as the material of facial prostheses, master models are fabricated by RP. In the approach, data on a facial prosthesis for a defective part are usually created by performing the mirror copy of the data on normal part. Figure 7 shows an example of the mirror copy which we performed to make a finger prosthesis. We use the 3D scanner, VIVID (KONICA MINOLTA), for obtaining the data on a normal finger, and we use 3D software, Rapidform (INUS), for performing the mirror copy. Ueda et al. apply 3D printing using starchy powder to make a master model for a facial prosthesis of auricle of ear (Ueda et al., 2004). In this work, CT is used for obtaining the data on normal auricle of ear. Sykes et al. compare the accuracy, required time, and potential advantages of RP technology with traditional methods in the manufacture of wax patterns for two facial prostheses (Sykes et al., 2004).

As other applications of RP, an occlusal splint is fabricated by SLA, in which bio-compatible acrylic resin is used (Witkowski, 2005). Other types of splints may be fabricated in the same way. RP is also used to fabricate individualized trays for indirect bracket bonding after they are designed by a CAD system (Ciuffolo et al., 2006).



3D model of a normal finger

3D model for fabricating a finger prosthesis for a defective finger

Figure 7:
Mirror Copy of 3D model

APPLICATION TO SURGERY

Maxillofacial Surgery

Physical maxillofacial models of human anatomy have been fabricated by RP from medical image data. This application is widely found in many countries. SLA and FDM have been predominantly used for the fabrication. Figure 8 shows an example of the mandible model which we fabricated using EDEN (OBJET). CT is usually used for making the image data, although MR and ultra-sound have also been used.

The physical maxillofacial models can be used (Winder et al., 2005):

- to aid production of a surgical implant;
- to improve surgical planning;
- to act as an orienting aid during surgery;
- to enhance diagnostic quality;
- to be useful in preoperative simulation;
- to achieve patient's agreement prior to surgery; and
- to prepare a template for resection.

Figure 8:
An example of mandible model fabricated using EDEN (OBJET)

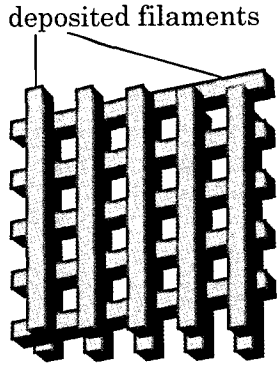


Figure 9:
Uniform porosity created by fused deposition modeling

Artificial bones as well as the physical maxillofacial models are sometimes fabricated by RP. In the conventional method of fabricating the artificial bones, a hydroxyapatite block is cut and modified during surgery to fit it into the diseased part of a patient. Since it is difficult to cut and modify the hydroxyapatite block, this tasks surgeons. To reduce the task, 3D printing is used to fabricate the artificial bones using the powder (Anzai et al., 2004; Kobayashi et al., 2004). Since the artificial bones made from hydroxyapatite may be broken after the surgery, SLS is also applied to fabricating the artificial bones made from titanium to avoid the breakage (Kobayashi et al., 2004).

Dental Implant Surgery

Physical maxilla and/or mandible models are fabricated by RP from CT data for dental implant surgery (Ishida et al., 2007). The models are used for simulating the insertion of implants before the surgery. RP is also applied to fabricating surgical guides, which are attached to patients and orient surgeons during the surgery (Kizu et al., 2007). SLA is often used for this purpose. NobelGuide provides such surgical guides after planning the insertion of implants on computers and sending the data through internet. Some other works have been carried out for the surgical guide fabrication by RP.

APPLICATION TO TISSUE ENGINEERING

Scaffold Fabrication

Tissue engineering aims to produce biological substitutes for damaged tissue or organs. This approach involves the fabrication of scaffolds, which provide a temporary template for the growth of target tissues, and transplantation of cells onto them. A successful scaffold should possess the following characteristics (Leong et al., 2003):

(i) a suitable macrostructure to promote cell proliferation and cell-specific matrix production;
(ii) an open-pore geometry with a highly porous surface and microstructure that enables cell ingrowth;
(iii) optimal pore size employed to encourage tissue regeneration and to avoid pore occlusion;
(iv) suitable surface morphology and physiochemical properties to encourage intracellular signaling and recruitment of cells; and
(v) being made from a material with a predictable rate of degradation, with a nontoxic degraded material.

In addition to these, suitable mechanical strength is required in some cases.

Since it is difficult for the conventional removal fabrication methods to fabricate freeform scaffolds with inner structure, a lot of studies on the application of RP techniques have been carried out to fabricate such scaffolds.

FDM is most often used for fabricating the scaffolds. The porous structure can be produced by depositing filaments extruded from the nozzle, as shown in Fig. 9. FDM can control the form and size of pores by manipulating the movement of nozzle. Moreover FDM can deal with various materials including nontoxic degradable ones. These are the reasons why FDM is often used. Polycaprolactone (PCL) scaffolds with a honeycomb structure (Zein et al., 2002), polymer-ceramic composite scaffolds made of polypropylene-tricalcium phosphate (PP-TCP) (Samar et al., 2003), and PCL-hydroxyapatite (HA) scaffolds (Endres et al., 2003) were fabricated by FDM. To make FDM more suitable for the scaffold fabrication, the following studies have been carried out:

(a) to reduce operating temperature of FDM for incorporating biomolecules into scaffolds; and
(b) to input material in pellet form instead of the filament in order to enhance the range of materials.

3D printing has also been applied to fabricating the scaffolds with various materials. Polylactic-co-glycolic acid (PLGA) mix with salt particles and a suitable organic solvent (Kim et al., 1998), a blend of starch-based polymer powders (Calvert, 2001), PLLA

(Zeltinger et al., 2001), etc. have been tried to use for fabricating the scaffolds. In addition to the macroporosity in the scaffolds, 3D printing can create the microporosity that arises from the space between the individual granules of powder in the scaffolds.

Though SLA is a well-known RP technique, it is difficult for SLA to fabricate the scaffolds because of the restriction on materials that SLA can handle. However there are several researches that attempt to use nontoxic biodegradable materials (Niino, 2007).

There is another type of approaches that produce the scaffolds indirectly; that is, the approaches producing molds or master models for fabricating the scaffolds. Most of RP techniques including SLA can be applied for this purpose.

Bioprinting

Bioprinting is defined as computer-aided, automatic, layer-by-layer deposition of biologically relevant materials (e.g., molecules, cells, tissues, and biodegradable biomaterials) with a prescribed 3D organization to accomplish one or more biological functions (Mironov et al. 2006). There are several researches including a bioprinting project in Japan. In the short term 2D printed cell assays is a realistic goal, however in the medium and long term bioprinting of 3D tissues and organs is a challenging but worthwhile pursuit (Mironov et al. 2006).

CONCLUSIONS

The authors outlined the types of RP and surveyed the various applications of RP in dentistry, which include: fabrication of dental and facial prostheses; oral and maxillofacial surgery; and tissue engineering. Different types of RP technologies are applied, depending on the purposes of applications. However it has not been enough to study which RP technology and which material are the most suitable for each purpose, taking account of various viewpoints including cost, time consumption and functionality. In addition, the comparison between the RP technologies and the conventional fabrication methods have not been studied enough with regard to their applications to dentistry. Nevertheless, RP is important in this area since RP enables us to fabricate complex objects that the conventional methods can not fabricate.

Lastly we would like to insist that RP serves as an interface between dentistry and technology. In other words, RP becomes a bridge between dentists and dental technicians (or engineers). We hope that the dental technicians will not only handle RP but also carry out the research on RP and play an important role in clinical teams.

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Hyper Human Technology and Its Applications

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ABSTRACT

This paper describes the development of a hyper human vision system, H^3 Vision, for realizing high-speed realtime image capture and processing, which are considerably beyond that capable by the human eye. This system has an exceptionally fast frame rate of above 1000 fps and an excellent spatial resolution of up to 1024×1024 pixels. Applications of this system to robotics, multimedia, and biomedical fields are also introduced, and the effectiveness of high-speed vision as one of the hyper human vision technologies is discussed.

I. INTRODUCTION

Most of the conventional image recognition technologies involving video signals (NTSC 30 fps / PAL 25 fps) are designed based on the characteristics of the human eye. Similarly, the processing speed is limited to the same or a lower level as compared to the human eye. In various fields of application such as factory automation, multimedia, and biomedical fields there is a growing demand for imageprocessing technology, which facilitates the real-time recognition of high-speed phenomena that cannot be recognized by the human eye.

Therefore, this paper describes a high-speed vision system as one of hyper human technologies for real-time image processing at a frame rate of more than 1000 fps. The core technology of the ultrafast hyper human technology initiative, which is being primarily led by the COE program on "Hyper Human Technologies Toward the 21st Century Industrial Revolution" at Hiroshima University, is introduced together with the applications of this system in the fields of mechanical control, multimedia, biomedicine, etc.

II. H^3 VISION SYSTEM

To realize a hyper human vision system featuring an exceptional spatial resolution and a speed considerable higher than that of the human eye, we developed a high-speed vision platform, H^3 Vision (Hiroshima Hyper Human Vision) system that can process 10-bit color/gray images with a resolution of 1024×1024 pixels at 1000 fps and images with a resolution of 256×256 pixels at 10000 fps in real time. As Fig. 1 shows, the H^3 Vision system consists of a high-speed camera head with a color/gray CMOS imager with a resolution of 1024×1024 pixels, a dedicated fieldprogrammable gate array (FPGA) board, and a personal computer (PC).

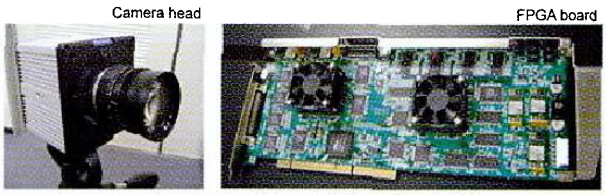


Fig. 1. High-speed vision platform, H^3 Vision

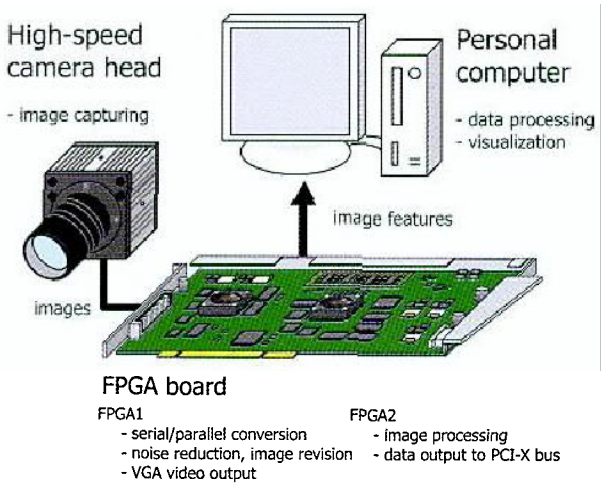


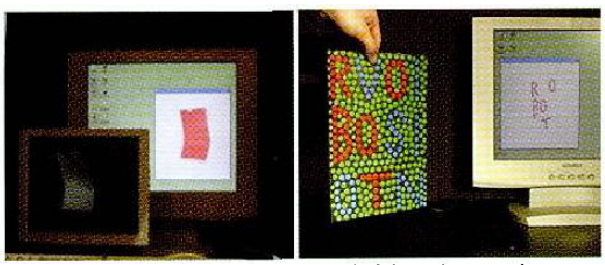
Fig. 2. High-speed real-time visual processing by H^3 Vision

Fig. 2 shows the basic processing flow of a H^3 Vision system. For image processing, the high-speed camera head can capture images at a set frame rate and spatial resolution and obtains the image data. An enormous volume of image information, exceeding 1 Gbytes/s, is transferred to the dedicated FPGA board by high-speed serial communications. In the hardware, during real-time image processing, the FPGA board processes all the pixels of the images to compressed image features such as moment features correlated to the object's size, position, orientation, and other parametric values required for image recognition. The processed information is then transferred to the PC memory through a PCI-X bus. Based on the transferred image features, the PC completes the final processing and displays the processed results.

TABLE
SPECIFICATIONS OF THE DEDICATED FPGA BOARD

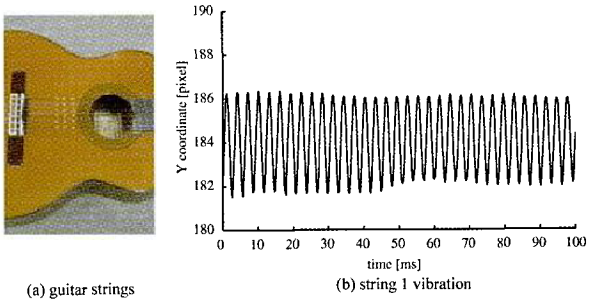
Board size	312 mm × 128 mm
Camera I/F	digital serial 12 ch
FPGA	Xilinx XC2VP100 × 2
Memory	DDR-SDRAM 640 MByte SDRAM 512 MBytes SSRAM 9 MBytes FPGA internal memory 999 kBytes
Bus I/F	PCI-X (64 bits, 66 MHz)

The dedicated FPGA board has two FPGA devices (Xilinx XC2VP100: FPGA1 and FPGA2) that the user can use to implement arbitrary circuits for image processing. Thus, the H^3 Vision system is not a specific processing system with limited features. Rather, it is a high-speed vision platform that can realize various kinds of image processing. Table I shows the specifications of the dedicated FPGA board. FPGA1 executes the serial-to-parallel conversion of the captured images, image correction (fixed-pattern noise removal and shading correction), and provides the external output of the VGA. FPGA2 performs user-installed arbitrary image processing on the image information transferred from FPGA1 through a 160-bit bus and also communicates with the PC via the PCI-X bus. The DDR-SDRAM memory, which is directly connected to FPGA2, can temporarily store the image information that is necessary for processing.



(a) White markers extraction (b) Color markers extraction

Fig. 3. Real-time multipoint marker extraction (1000 fps)



(a) guitar strings (b) string 1 vibration

Fig. 4. Real-time vibration measurement of guitar strings (330 Hz)

By implementing algorithms for different applications, the high-speed vision platform can be applied to various kinds of high-speed image processing. We actually realized the real-time multipoint marker extraction of 4000 points or more at 1000 fps and 254 points at 10000 fps by implementing a color extraction circuit, labeling circuit, and marker tracking algorithm in the H^3 Vision system. Fig. 3 shows multipoint color extraction at 1000 fps. The left-hand figure shows color marker extraction for 1024 points and the right-hand figure shows the result obtained when only red markers were extracted from a variety of colors. Thus the H^3 Vision system is also capable of position extraction of more than 1000 points and marker extraction with high associated accuracy using color information. Fig. 4 shows the time-series changes during the vibration of guitar strings (String 1 release: 330 Hz) measured by marker tracking at 10000 fps. We simultaneously measured the vibrations of the six strings and confirmed the agreement between the measurement results and the tuned frequencies. Based on these findings, the H^3 Vision system can even be employed to accurately measure vibratory phenomena and other ultrafast movements that cannot be observed by an unaided human eye.

As shown in this example, the H^3 Vision system is a highspeed vision platform equipped with both high-speed realtime capabilities and a high spatial resolution. Consequently, the system is expected to become a powerful measurement and analysis system in a variety of fields, including factory automation, robotic, multimedia, biomedical, and biological fields. From here, this paper introduces applications of the high-speed vision technology.

III. APPLICATION TO THE ROBOT CONTROL FIELD

This section introduces some of the applications of the high-speed vision technology to a mechanical system.

To realize a robot system for batting faster than a human, we developed a batting robot by combining high-speed vision units and a high-speed manipulator. The batting robot instantaneously predicts the time and course of a ball at the batting position using the two high-speed vision units operating at 1000 fps and swings the bat at a high speed using the high-speed manipulator according to the predicted information. In fact, the robot successfully hit the balls with a speed of 160 km/h that were pitched from a pitching machine. Fig. 5 shows the high-speed batting experiment. The high-speed manipulator of the robot is positioned 6.7 m away from the cameras and hits the balls according to the calculated and predicted 3D trajectory information. Thus, the robot realized high-speed manipulation that was beyond the capability of humans.

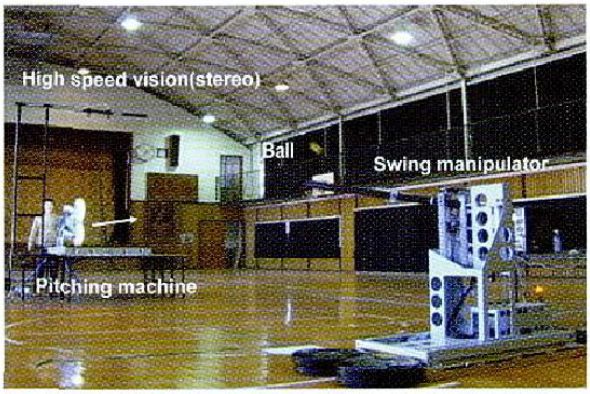


Fig. 5. A 160 km/h ball batting experiment

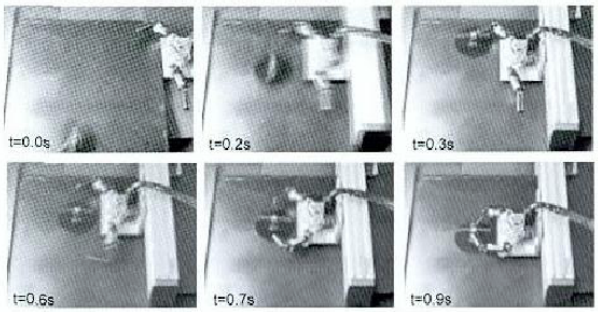


Fig. 6. Capturing a bar-shaped object by a robot hand

In another robot control test, we successfully developed a robot that captured the movement of a bar-shaped object with high-speed rotation and translated the movement in real time with high resolution by using the high-speed vision technology. Fig. 6 shows the images obtained while capturing the bar-shaped object. Based on visual feedback information, the robot grasps the object using its hand

It is also predicted that the introduction of this high-speed vision technology will realize high-speed handling by means of a mechanical control technique, although this technology is not yet considered feasible. The applicable manipulation technology using high-speed visual feedback is expected to develop rapidly, particularly in the factory automation field that has witnessed considerable development in recent years.

IV. APPLICATION TO THE MULTIMEDIA FIELD

For applications involving motion capture, human actions, and interactions with the real environment, high-speed vision can be employed to measure and recognize movements faster than the unaided human eye.

We created a fingertip-tapping interface that extracts highfrequency oscillating components generated by fingertip tapping using the high-speed vision. Fig. 7 shows the results of frequency conversion of fingertip motions in the vertical direction when a subject's index finger is quickly and repeatedly moved up and down. The solid line and dotted line represent the data collected during contacts and in the absence of contact, respectively. We can observe the high frequency components that are greater than 40 Hz only when there are contacts; this implies that a high-speed vision system can also detect and utilize the points of contact. Fig. 8 shows a virtual musical instrument that employs this interface system. When the instrument is touched, this system generates a scale, volume, and timing according to the position and estimated force of contact in order to give the player the impression of playing a piano.

We have also developed a 3D measurement system for capturing the movement of an object using numerous markers by adding a space conversion optical system to the H^3 Vision system. This system measures the 3D translation and rotation of an object in real time using hundreds of markers. Fig. 9 shows the movement of the ball captured with the high-speed camera, and Fig. 10 shows the results of the real-time motion measurement at 1000 fps. A table-tennis ball with a diameter of 40 mm, rolled at 2.5 m/s and at 8 rps is used in this study. These measurements prove the effectiveness of high-speed vision for multipoint 3D motioncapturing at a high speed in real time.

We have also developed other applied systems such as a gesture robot for the game of "paper, stone, scissors" that can always beat humans, as shown in Fig. 11, and proved that the high-speed vision technology capable of measuring and recognizing movements beyond the capturing capacity of the human eye is effective in the multimedia field.

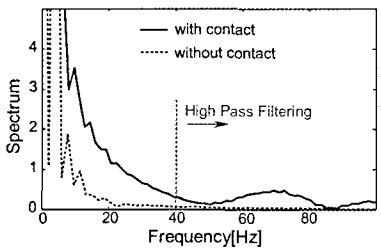


Fig. 7. Motion frequency in finger tapping

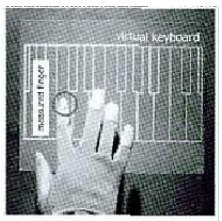


Fig. 8. Virtual musical instrument by fingertip-tapping interface

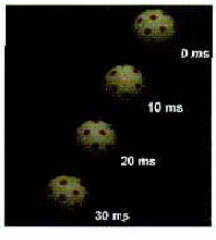


Fig. 9. Translation and rotation of a ball (captured by a high-speed camera)

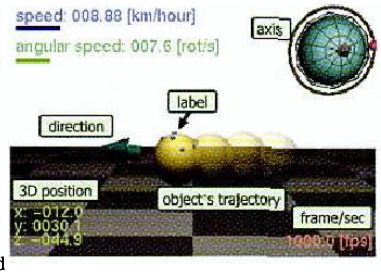


Fig. 10. Real-time motion measurement of a ball

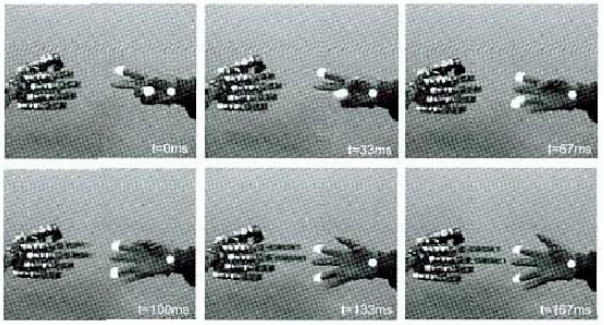
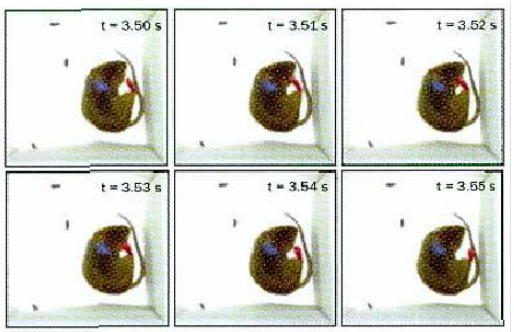


Fig. 11. A gesture robot for the game of "paper, stone, scissors"

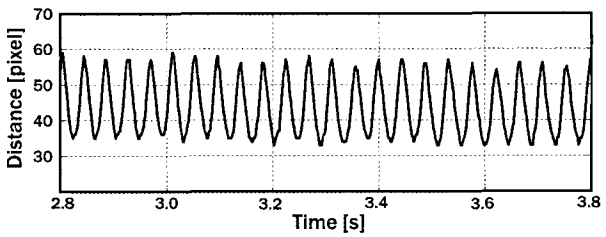
V. APPLICATION TO THE BIOMEDICAL FIELD

In addition to the measurement and analysis of human motion, high-speed vision may also be applied to the biomedical field as an advanced measuring system on various scales.

A laboratory mouse scratches its head repeatedly using its hind leg at a frequency of approximately 20 times/s, which is too fast for humans to observe. However, using high-speed vision, users can detect changes in the image features from the variations in the distance between the hind leg and the head. Thus, accurate measurements of the scratching frequency and period of scratching for the mouse can be obtained for use as a behavioral evaluation index for atopic dermatitis. Fig. 12 shows an example of the scratching movement of the mouse. The *in vivo* evaluation system for quantifying disease-related behaviors of laboratory animals is being put to practical use as an effective and efficient evaluation system for new medicines for atopic dermatitis.

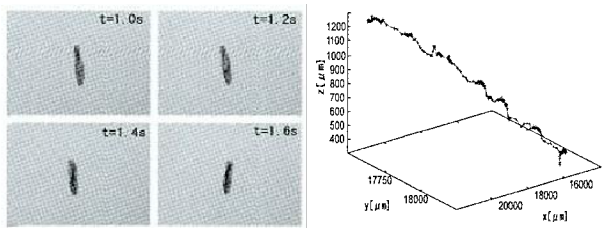


(a) High-speed video images in scratching



(b) Head-toe distance in scratching

Fig. 12. Scratching movement of a laboratory mouse



(a) monitor image (b) 3D trajectory

Fig. 13. 3D Tracking of swimming paramecia

The microscopic measurements of moving objects are often very fast and erratic. In such instances, high-speed vision is extremely effective for the real-time measurement and analysis of the movement of the objects. We have realized a real-time 3D tracking system for a microscopic object based on the information obtained from high-speed microscopic observations of paramecia, which measured approximately 200 im, as shown in Fig. 13. The real-time, high-speed measurement of movements on a variety of scales is highly effective for the analysis of biological behaviors, which are important phenomena.

The real-time measurement technology that employs highspeed vision can be applied to rigidity measurements of the response of the eyeball and skin to an imposed force and to the establishment of a new diagnostic technique based on the real-time biological measurement information acquired by the high-speed vision system. The technology is likely to benefit the medical field considerably.

VI. CONCLUSION

This paper describes the next-generation hyper human vision system, H^3 Vision system, for real-time processing at a rate of 1000 fps or more. Several applications are also introduced to demonstrate the considerable potential of highspeed vision in various fields that lie beyond the human capability.

Automutanolysin (Aml), a Novel Bactericide Targeting Cariogenic Streptococci

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ABSTRACT

Automutanolysin (Aml) is a recently identified autolysin produced by *Streptococcus mutans*. Aml has a modular design where the N-terminus contains five 13-amino-acid repeats which presumably act as cell wall targeting domain and a C-terminal enzyme active domain. Aml selectively lyses *S. mutans* and *S. sobrinus* but no other oral streptococci. This suggests Aml possesses strong substrate specificity towards cariogenic bacteria present in the human oral cavity. Analysis of *S. mutans* peptidoglycan fragments released by Aml shows the enzyme acts as an *N*-acetylmuraminidase. Aml is active against not only planktonic cells but also those forming biofilm. These characteristics suggest that Aml can be a possible candidate as a bactericide selectively targeting the cariogenic streptococci.

INTRODUCTION

Peptidoglycan hydrolases have been proposed that they are involved in cell wall metabolism including cell growth, cell division, separation, cell-wall turnover and also pathogenicity. According to the chemical bond of the peptidoglycan substrate that these enzymes digest, they are classified as endo-β-*N*-acetylglucosaminidase, endo-β-*N*-acetylmuraminidase, *N*-acetylmuraml-L-alanine-amidase, endopeptidase and transglycosylase. Some enzymes have an ability to digest their own peptidoglycan, when bacteria are exposed to antibiotics and other harmful conditions; and such enzymes are called autolysins (1,7,9,12).

Streptococci are Gram-positive bacteria where individual cells are connected with each other forming a chain morphology. Among the streptococci, mutans streptococci has clear association with dental caries. Although dental caries is not a life endangering disease, they are linked to general health, well-being and quality of life. The World Health Organization (WHO) confirms that dental caries is a major public-health problem, owing to its high prevalence in all regions of the world (8). One of virulence factors of *S. mutans* is the formation of biofilm (dental plaque) that have been implicated in increased resistance to antibiotics, antimicrobials and host response, as the dense extracellular matrix and the outer layer of cells protect the interior of the community (2,3,6,10).

We have demonstrated that *Streptococcus mutans* produces two bacteriolytic enzymes of 80 kDa and 100 kDa (13). We identified the proteins corresponding to these enzymes and found that they are from a single gene product (14). We called this enzyme Aml for automutanolysin. Aml selectively lyse *S. mutans* and *S. sobrinus* but not other oral streptococci. This suggests Aml possesses strong substrate specificity towards human cariogenic bacteria that may be exploited as an alternative means for caries prevention.

In this literature, we will briefly review characterization of Aml and discuss its potential as a bactericide targeting cariogenic streptococci.

RESULTS AND DISCUSSION

Identification of 80 and 100 kDa bacteriolytic enzymes. We previously showed that *S. mutans* produces two major bacteriolytic bands at 80 and 100 kDa using zymographic analysis (13). N-terminal sequence of two proteins was VQARSSLTQD for the 80 kDa band and DEQNQSLSAS for the 100 kDa band. The sequence was compared to the TIGR Microbial Genome Database for *S. mutans* UAB159 using the BLAST program. This identified an ORF consisting of 2,937 bps coding for a hypothetical protein of 979 amino acid residues with an apparent molecular weight of 107 kDa. We named this ORF protein Aml. The deduced N-terminal sequence of the 100 kDa protein shows Aml possesses a signal peptide characteristic of a secreted protein. The first 23 amino acid residues of Aml appears to correspond to a signal peptide that has a cleavage site between S_{23} and D_{24} according to the criteria as described previously (11). The processed protein starting at V_{167} has a calculated molecular weight of 89.7 kDa. This suggests the 80 and 100 kDa proteins are coded on the same ORF and the 80 kDa protein is an N-terminally processed form (Fig. 1A).

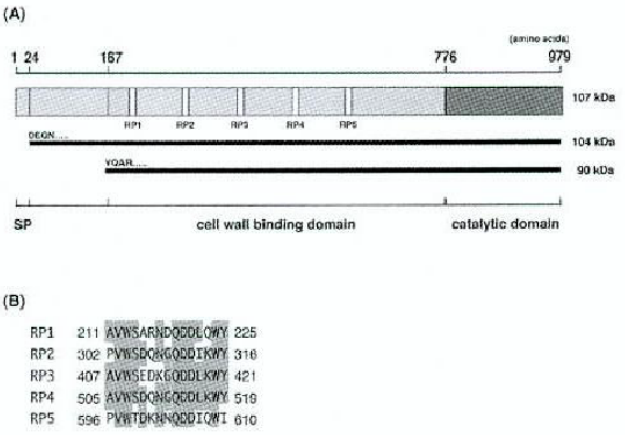

Fig. 1. Schematic representation of the primary sequence of Aml. (A) Aml is composed of the two regions, the putative cell wall banding domain and catalytic domain. RP and SP represent the repeat region and signal sequence, respectively. (B) Alignment of the 5-repeat regions. Repeat regions (RP1-5) at the putative cell wall binding region are aligned and similar amino acids were indicated using a gray backgrounds.
h Aml (A) and mutanolysin (B), and the digests were subjected to HPLC analysis.

Analysis of Aml structure. Self-alignment of the deduced sequence of Aml shows the presence of five repeats (RP1-RP5) of 15 amino acid sequences at the N-terminal end (Fig. 1B). Similarity search for Aml with the predicted coding sequence present in the public databases using the PSI-BLAST, BLAST and FASTA algorithms was performed and suggests Aml is composed of two modular structures, the N-terminal (25-776) and C-terminal (776-979) modules. The C-terminal portion of Aml shows high similarity to the enzyme active domains of several Lysozyme M1s (1,4-b-*N*-acetylmuraminidase), endolysin of *L. helveticus* phage f-0303 and prophage Lp2 protein 56 lysin of *Lactobacillus plantarum* WCFS1 (CAD64709) whereas the N-terminal portion shows similarity to various proteins with different functions including LPXTG cell wall surface anchoring protein of *S. aureus* (YP187464) and mucin 4. Most of bacteriolytic enzymes possess a two-domain structural organization. One portion has enzymatic activity while the other is responsible for substrate-binding. A variable number of repeated sequences in the substrate binding domain have been demonstrated to play a direct role in cell wall binding. We therefore expect that the 5 repeats of the 15 amino acid span in the N-terminus of Aml may play a role in peptidoglycan binding. However, this region shows no similarity to any characterized cell wall binding motifs. Thus, the function of the N-terminus remains to be determined.

To determine the region necessary for lytic activity, several truncated and point mutant recombinant Hisx6-tagged Aml were constructed and evaluated for their ability using zymography. Hisx6-tagged full length Aml and N-terminally truncated Aml show bacteriolytic activity, but the activity of the putative catalytic domain alone was significantly lower where the lytic band could not be seen in a zymogram. The amino acid substitution of D_{869}, an essential amino acid in the active site of lysozyme M1, to A completely abolished the bacteriolytic activity of both full length and N-terminally truncated Aml. This further confirmed that the C-terminus possesses the catalytically active site.

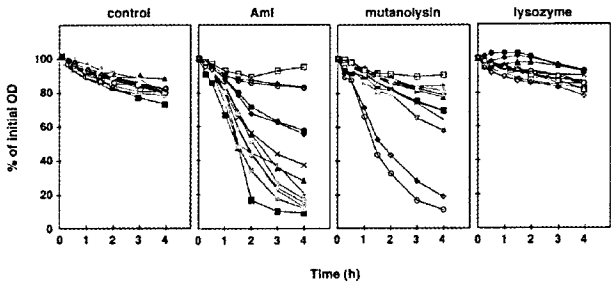

Fig. 2. Lytic activity of Aml against oral streptococci. Viable cells from 5 *S. mutans*, 5 *S.sobrinus* and 3 *S. salivalius* strains were exposed to 10 μg/ml Aml, mutanolysin or lysozyme. Bacterial cell lysis was measured at OD_{660nm}. Symbols: ♦, *S. mutans* 403R; ■, *S. mutans* 175; ●, *S. mutans* C67-1; ▲, *S. mutans* LM-7; ×; *S. mutans* 703R; ♦, *S. sobrinus* 6715; ⊠, *S. sobrinus* B13; ⊛, *S. sobrinus* OMZ176a; ⁂, *S. sobrinus* 615R; ×, *S. sobrinus* SL-1; ◇, *S. salivarius* H665; □, *S. salivarius* 9222; and ○, *S. salivarius* H216.

Identification of the 100 kDa bacteriolytic enzyme as muraminidase. Purified peptidoglycan from *S. mutans* OMZ175 was incubated with the purified Hisx6-tagged full length Aml. The appearance of free amino groups and reducing sugars during enzymatic hydrolysis was monitored. An increase in the concentration of reducing sugars together with the decrease in turbidity was observed and suggests Aml is a hexosaminidase. We further analyzed the reducing sugars that increased after Aml-digestion to determine the enzyme specificity. The peptidoglycan sample with or without Aml-digestion was treated with alkali-NaBH4 to convert the reducing end of *N*-acetylhexosamine to *N*-acetylhexosaminitol, followed by complete acid hydrolysis. HPLC analysis of the phenylthiocarbamyl derivatives of hydrolyzed sample shows an increase in *N*-acetylhexosaminitol together with a decrease in *N*-acetylmuramic acid in Aml-digested peptidoglycan; while the amounts of *N*-acetylglucosamine and *N*-acetylglucosaminitol were not altered. These results show Aml is acting as an *N*-acetylmuraminidase. We compared the HPLC pattern of the *S. mutans* peptidoglycan-digest by Aml to that of a digestion by mutanolysin, a commercially available muraminidase. The fact that patterns of Aml digest and mutanolysin digest are indistinguishable further support Aml is an *N*-acetylmuraminidase.

Substrate specificity. The lytic activity of Aml was assessed using viable cells of *S. mutans, S. sobrinus, and S. salivarius.* As shown in Fig. 2, His-Aml preferentially lysed the cells of *S. mutans* and *S. sobrinus* but not *S. salivarius.* The lytic activity of His-Aml was compared to that of other commercially available lytic enzymes: egg-yolk lysozyme and mutanolysin. As shown in Fig. 2, mutanolysin showed relatively weak lytic activity against most oral streptococci tested and strong activity for *S. salivarius* H665 and H216; whereas, lysozyme showed almost no activity. This suggests His-Aml has substrate specificity and preferentially lyses two major cariogenic pathogens.

To further characterize the substrate selectivity, we examined viable cells, heat-inactivated cells and purified peptidoglycan from various oral streptococci. As shown in Fig. 3, His-Aml showed substrate selectivity towards *S. mutans* and *S. sobrinus* when peptidoglycan was used as the substrate indicating that Aml can differentiate the peptidoglycan structures of the oral streptococci.

It is not surprising that Aml digests the peptidoglycan of *S. mutans* since it is the producer of the Aml. However, it was unexpected that *N*-acetylmuraminidase showed substrate selectivity although the substrate glycan chain is ubiquitously present in all bacterial peptidoglycans. Aml may recognize not only the glycan chain but the complex structure of *S. mutans* and *S. sobrinus* specific peptidoglycans. The detailed mechanism how Aml differentiates the peptidoglycan of *S. mutans* and *S. sobrinus* from other oral streptococci remains to be determined.

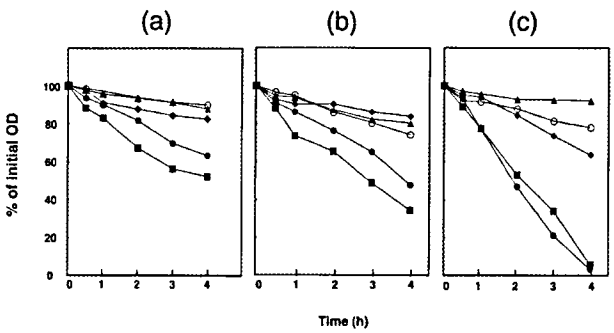

Fig. 3. Lytic activity of Aml against five viable cell strains (a), heat-inactivated cells (b) and purifed peptidoglycan (c). The turbidity of the cell suspension was measured at OD_{660nm} after the addition of Aml (10 μg/ml). Symbol; ■, *S. mutans* C67-1; ●, *S. sobrinus* OMZ176a; ▲, *S. sanguinis* ATCC10556; ♦, *S. mitis* ATCC9811; ○, *S. salivarius* .

Lytic activity on oral clinical streptococci. We examined its lytic ability against live clinically isolated cariogenic streptococci. We first examined enzyme activity on planktonic cells. We have previously demonstrated that non-ionic detergent stimulates lytic activity of autolysin (5). Preliminary experiment demonstrated that 0.1% of Triton X-100 provided maximum stimulating effect. We therefore used Aml preparation containing 0.1% Triton X-100 for further assays. Aml showed lytic activity towards the clinical strains although the enzyme susceptibility varied among the strains, and further confirmed that Aml is active against cariogenic bacteria. Surprisingly, one strain, *S. sobrinus* 5, showed complete resistance to this enzyme. The detailed mechanism how *S. sobrinus* 5 is resistance to Aml remains unknown, but a possibility of modification within the peptidoglycan structure must be investigated in the future study.

In an oral environment, most bacteria are present in biofilm. They encase themselves in a hydrated extracellular polymeric substances matrix that protects them from a harmful environment (2,3,6,10). It is possible to assume that cells in biofilm are less susceptible than those in planktonic form to lysis by exogenously added bacteriolytic enzyme due to hindrance by matrix architecture. We asked if Aml can penetrate biofilm and lyse bacterial cells in situ. Biofilm cells of clinical oral streptococci were treated with lysis buffer containing His-Aml in the absence or presence of Triton X-100, and the supernatant of the lysis buffer was probed for the bacterial DNA by PCR. The amount of DNA was determined by measuring the fluorescence intensities of the stained bands with an ATTO image analyzer and NIH image software. His-Aml treatment in the absence or presence of Triton X-100 induced the appearance of PCR amplicon of *dexA* gene. This indicated the ability of Aml to lyse bacterial cells in biofilms. We next examined whether repeated rounds of Aml treatment on biofilm cells can degrade biofilm matrix architecture. Repeated rounds of His-Aml treatment in the presence of Triton X-100 is clearly effective against biofilm of tested strains.

These results together suggested that Aml is a potential candidate for bactericide selectively targeting cariogenic bacteria. The challenge for the future would be improvement of lytic activity and animal experiment to prove its potential to eradicate cariogenic streptococci in vivo.

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Oral Stereptococci May Kill Pathogens in the Oral Cavities in Cooperation with Secretory Immunoglobulin A

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ABSTRACT

Early colonization of Viridans streptococci significantly reduced the colonization of methicillin-resistant *Staphylococcus* aureus (MRSA) in newborns' oral and nasal cavities. Hydrogen peroxide (H_2O_2) produced by >500 viridans streptococci cells killed 1 cfu MRSA cell in vitro. Secretory IgA (SIgA) in saliva and colostral SIgA bound *Streptococcus sanguinis*, a viridans streptococcus, and MRSA into coaggregates. H_2O_2 produced by *S. sanguinis* in the coaggregates was decomposed by salivary peroxidase and bacterial catalase, and a short lived by-product, singlet molecular oxygen ($^1O_2$) was synthesized. The SIgA converted $^1O_2$ into ozone, which has a potent bactericidal and fungicidal activity. We calculated that <10 cfu of *S. sanguinis* were necessary to kill 1 cfu of MRSA in the coaggregate. SIgA, *A. niger* catalase, and H_2O_2 in saliva killed *Candida albicans* which is highly resistant to reagent H_2O_2. We propose that together with viridans streptococci and innate immunity, SIgA potentially constitute a novel system that may sustain oral homeostasis.

Key words: Viridans streptococci, H_2O_2, Singlet oxygen, SIgA, Salivary peroxidase.

INTRODUCTION

Despite extensive prevention efforts, methicillin-resistant *Staphlococcus aureus* (MRSA) colonization and infection has been a serious problem in many hospitals world wide. Furthermore, effective measures for treating MRSA infection would be lost in the near future, because of the MRSAs' acquisition of much potent resistant ability against antibiotics. This situation led us to study natural prevention measures.

Corynebacteria, normal bacterial flora, interfere with MRSA and eradicate it in the nasal cavities (*1*). Early colonization by viridans streptococci prevents oral cavity of a newborn infant from being colonized by MRSA (2). Bacterial interference between pathogens and normal flora may be responsible for this colonization inhibition, although the mechanism was not clearly identified yet.

Hydrogen peroxide (H_2O_2) produced by viridans streptococci kills MRSA in vitro (*3*). However, a single viridans streptococci cell produces insufficient H_2O_2 to kill MRSA that has strong catalase activity on its surface; concentration or augmentation of the bactericidal activity of H_2O_2 is necessary for killing of MRSA in oral cavity. Coaggregation between MRSA and viridans streptococci may compensate for the inadequate amounts of H_2O_2. Secretory IgA (SIgA) binds to viridans streptococci non-specifically and binds to pathogens specifically via their N- and O-glycan and 4 Fab binding sites respectively (*4,5*).

If salivary SIgA contributes to form coaggregation in saliva, ozone molecule (O_3) may be produced by immunoglobulin-catalyzed water-oxidation reaction from singlet oxygen ($^1O_2$), products of H_2O_2 degradation reaction by salivary peroxidase (*6-8*). Since the ozone molecule has a very strong microbicidal activity, many pathogens, even the ones resistant to H_2O_2, are possibly killed by this substance.

In the present review, we firstly examine bacterial interference between viridans streptococci and MRSA in newborn babies and the mechanisms of them. Next, we study the mechanisms of coaggregation formation, effect of the coaggregation on MRSA killing by H_2O_2, singlet oxygen production from H_2O_2 and interference of this singlet oxygen production by MRSA catalase. Finally we examine whether SIgA in the presence of saliva could promote the Ig-catalyzed water-oxidation reaction.

MATERIAL AND METHODS

Epidemiological study of the incidence of MRSA isolation in NICU.

Prospective surveillance cultures for all newborns were first performed at the time of admission (when the newborn is <24 h of age), every subsequent Monday during each patient's entire hospitalization, and at 1 day before discharge. According to clinical requirements, cultures were occasionally performed between the prospective cultures (2).

Assay of the bacteriocin-like activity of viridans streptococci.

Microorganisms (1×10^6cfu) in 100 μl of PBS was mixed with 10 μl of melting brain-heart infusion broth with 1.5% low-melting-point agar and poured into a plastic dish. After solidification, the mixture was overlaid with 10 μl of the same medium without microorganisms. Suspensions of viridans streptococci (1×10^7 cfu) in 10 μl of PBS were spotted on the agar plate and incubated overnight at 37℃. After incubation, diameters of inhibition were measured (*3,9*).

Aggregation and coaggregation.

Viridans streptococci (*Streptococcus sanguinis* ATCC10556^T, *S. gordonii* ACC10558T, *S. oralis* NCTC1142 7^T, *S. mitis* NCTC12261^T), MRSA (clinical isolates and catalase-negative *Staphylococcus aureus* TW4632, unstained or stained with ethidium bromide), or both were mixed, on a glass slide for several minutes, with either intact saliva, IgA- or IgG-depleted saliva, or colostral SIgA, and the aggregates were observed macro- and microscopically (*9*).

2-Dimmensional gel electrophoresis, and analysis by matrix-assisted laser-desorption time-of-flight mass spectrometry (MALDI TOF MS) analysis.

Proteins were analyzed according to the method described by Yao et al. (*10*). *S. sanguinis* cell-sediments were incubated with saliva. Salivary components bound to the cell surface were extracted with 4 M NaCl. *S. sanguinis* proteins were extracted with 8 M urea-2 M thiourea-4% 3-[(cholamidopropyl) dimethylammonio]-1-propane sulfonate (CHAPS) (*11,12*). The solvents of these extracted proteins were substituted with PBS or 8 M urea by centrifugal filter device (Millipore, Bedford, MA, USA). *S. sanguinis* proteins bound to SIgA were prepared from *S. sanguinis* proteins using a colostral SIgA-coupled affinity column. These proteins were analyzed by 2D gel electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF MS) (Applied Biosystems, Foster City, CA, USA). Mass fingerprints were used to screen tryptic fragment libraries with Mascot (Matrix Sciences Ltd; www.matrixscience.com) and Protein Prospector (University of California, San Francisco; prospector.ucsf.edu) (*9*).

Singlet molecular oxygen analysis.

The method described by Wentworth et al. (*13*) was used. In wells of microtiter plate, 3 μl of indigo carmine (1mM), which reacts with O_3, $^1O_2$, and H_2O_3, was mixed with PBS (pH 7.4), 5 μl of MRSA in PBS at various concentrations (2.5×10^5, 1.0×10^6, 2.0×10^6, and 4.0×10^6), 1 μl of 1% H_2O_2, and 91 μl of PBS or saliva, and incubated at 37℃. The decrease in absorbance was measured at 600 nm at various time points using a microtiter plate reader. In some experiments, various amounts of *Aspergillus niger* catalase (0.03 to 300mU) (Sigma-Aldrich, St. Louis, MO, USA) or *Staphylococcus aureus* TW4632 (catalase-negative strain) were used instead of MRSA (*9*).

Ozone determination by 4-vinyl benzoic acid oxidation reaction.

The method described by Wentworth et al. was used to measure 4-vinyl benzoic acid oxidation (*13*). Saliva was mixed with 1 mM 4-vinyl benzoic acid, which reacts with O_3, in 150 μl PBS (pH 7.4), 30 μl 1% H_2O_2, and 1.1 mg human colostral SIgA in 20 μl PBS, and incubated at room temperature for 3 h. Twenty-microliter aliquots were removed and diluted 1:3 in acetonitrile:water (1:1). The product composition was determined by HPLC with a Shim-Pack CLC-ODS column. Products were detected with UV at 254 nm (R^T 4-vinyl benzoic acid=11.47 min, R_T 4-carboxybenzaldehyde=3.83 min, R_T 4-oxiranyl-benzoic acid=4.31 min). In the control assay, 4-vinyl benzoic acid was oxidized by irradiation on a transilluminator (312 nm, 0.8 mWc m^{-2}) at room temperature in the presence of SIgA, and the product composition was determined using a similar method (*9*).

Killimg of MRSA by S. sanguinis in a 50% saliva-agarose plates.

Microorganisms (3ml, 1×10^8 CFU) in PBS was mixed with 9ml of BHI broth (pre-incubated at 50℃ for 10min) containing 50mg of low-melting-point agarose and 6 ml saliva, and poured into a plastic plate. After solidification, 3.3mg of colostral SIgA (20 μl) was spotted at three independent points on the plate and allowed to soak into the gel, after which 1×10^8, 1×10^7, or 1×10^6 CFU *S. sanguinis* (ATCC10556^T) (10 μl) were spotted onto one of the SIgA-soaked points. The same amount of *S. sanguinis* solution was similarly spotted onto the plate at three points without SIgA. The plates were cultured overnight at 35℃ in 5% CO_2. After incubation, diameters of inhibition were measured (*9*).

Killing of C. albicans by H_2O_2 in 50% saliva-agarose plates

C. albicans (1×10^8 CFU) was embedded in saliva-BHI agar plates using the method described above, and colostral SIgA was soaked into the gel. *A. niger* catalase at 0.3 or 5.0 units was spotted onto SIgA-soaked points or onto two independent points with no SIgA. H_2O_2 (1%, 10 μl) was spotted onto the points soaked with SIgA, catalase, or both, or onto another point without SIgA or catalase. The plate was cultured overnight at 35℃ in 5% CO_2 (*9*).

Statistical analysis

Group data are expressed as mean ± SD, and differences between groups were analyzed by the paired Student's t test or the χ^2 test.

RESULTS

Interference in MRSA colonization mediated by viridans streptococci.

During the study period, cultures of specimens from 415 newborns in the NICU were performed. Total of 9699 specimens were obtained from nasal cavity, oral cavity, sputum specimens from bronchial aspiration, feces, breast milk, umbilical exudates, or other part of the body.

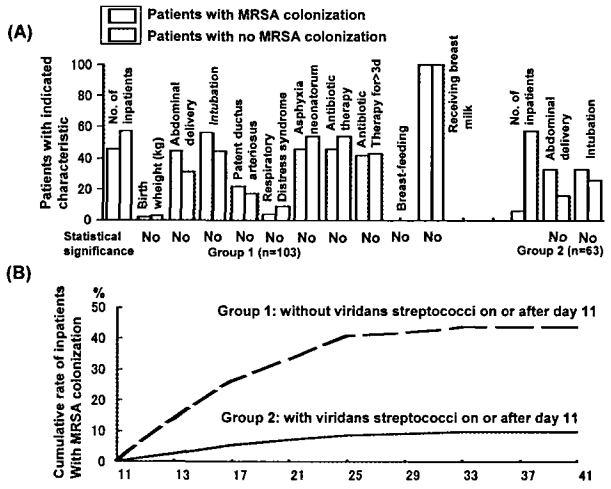

Figure 1.
Comparison of clinical characteristics of newborns without colonization with viridans streptococci (group 1 [103 inpatients]) and newborn inpatients with colonization with viridans streptococci (group 2 [63 inpatients]) on or afterday 11of hospitalization (*A*). No significant difference in characteristics was observed between the sub-groups. Comparison of later colonization with MRSA between the sub-groups (*B*). Forty-six of 103 group 1 newborns acquired MRSA colonization before discharge, while only 6 (9.5%) of 63 group 2 newborns acquired MRSA colonization. The rate of colonization was significantly different between the 2 groups (P<0.001).

Long-term hospitalization was one of the risk factors of MRSA colonization in the NICU. The rate of colonization with MRSA among newborns who were hospitalized for <11 days was comparatively low (17.3%), but among newborns who were hospitalized for >43 days, the MRSA colonization rate increased rapidly, and the number of newborns who were discharged without MRSA colonization decreased significantly. To reduce influences on the risk due to long-term hospitalization, 201 inpatients whose duration of hospitalization was 11-40 days were chosen for further study. Among them, 103 inpatients were not colonized with both MRSA and viridans streptococci (Group 1) and 63 were not colonized with MRSA, but with viridans streptococci (Group 2) on or after day 11. Group 1 of these groups was subdivided into 2 groups: 46 who acquired MRSA colonization and 57 who did not acquire MRSA colonization during hospitalization period. In a similar fashion, Group 2 was subdivided into 2 groups: 6 who acquired MRSA colonization and 57 who did not acquire MRSA colonization. The clinical characteristics (birth weight, abdominal delivery, intubation, patent ductus arteriosus, respiratory distress syndrome, blood culture-proven sepsis, asphyxia neonatoram, antibiotic therapy and breast feeding) of these subgroups were compared, however, no significant difference in characteristics was observed between the sub-groups (Fig 1A). Therefore, MRSA colonization of the nares and oral cavities did not seem to depend on any special characteristics of the newborns. The rate of MRSA colonization was much lower among the 63 Group 2 inpatients (6 [9.5%] of 63) than among the 103 Group 1 inpatients (46 [44.7%] of 103; p<0.001) (Fig 1B). Thus, colonization with viridans streptococci may counteract MRSA colonization.

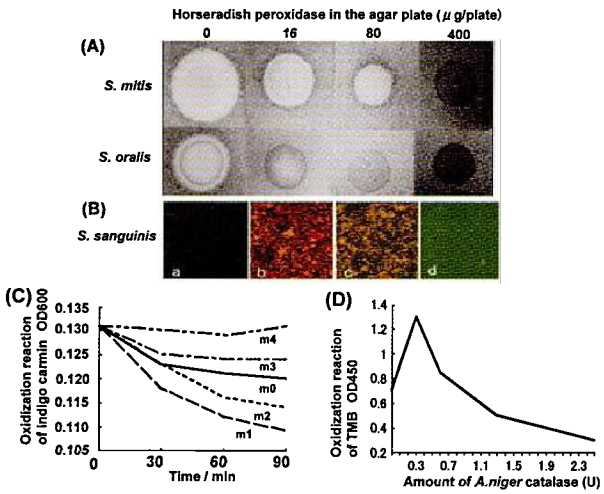

Figure 2.
Bacteriocin-like activity produced by viridans streptococci. BHI broth medium with low-melting-point agar containing horseradish peroxidase was poured on solidified MRSA, and an overlay was made; viridans streptococci were then spotted on it, and culture was performed. Viridans streptococci killed MRSA and created zones of inhibition (0 μg of horseradish peroxidase/plate). However, horseradish peroxidase neutralized the activity and reduced the diameter of the zone of inhibition in a dose-dependent manner (*A*). This bacteriocin-like activity was small enough to pass through a dialyzing-cellulose membrane separating two small chambers. MRSA (1×10^4 cfu) in one of the chambers was killed (*B*-a, acridine orange staining) by *S. sanguinis* in the neighboring chamber (*B*-b, survived). MRSA in a single-chamber device (*B*-c) survived; and MRSA cells killed by reagent H_2O_2 (*B*-d). Inhibitory activities of salivary peroxidase and microbial catalase to killing activity of H_2O_2, suspected effector molecule, were tested (*C*). In contrast to our expectation, salivary peroxidase decomposed H_2O_2 to produce more indigo carmine-reactive oxidant than was produced in the spontaneous degradation in PBS (C-m0). MRSA-catalase at smaller doses 2.5×10^5 cfu (line m1) and 1.0×10^6 cfu (line m2) increased the oxidant-producing activity of salivary peroxidase. MRSA catalase at larger doses 2×10^6 cfu (line m3) and 4×10^6 cfu (line m4), this activity was inhibited. *A. niger* catalase interfered with horseradish peroxidase in a similar manner (*D*). Small amounts of *A. niger* catalase (peak augmentation, 0,31 units; equivalent to the catalase activity of 5×10^5 cfu of typical MRSA) augmented the peroxidase activity.

Bacteriocin-like activity of viridans streptococci.

All viridans streptococci (18 strains tested) secreting H_2O_2 (1.24 ± 0.60mmol/1×10^8cfu/9h-culture in BH) inhibited MRSA growth on brain-heart-infusion agar and created zones of inhibition (Fig 2A). However, *S. salivarius, E. faecalis and S. epidermidis,* which lack the ability to secrete H_2O_2, did not have such activity. The amount of viridans streptococci necessary to completely kill 1 cfu of MRSA was >500 cfu. This bacteriocin-like activity produced by 1×10^7 cfu of viridans streptococci was completely inhibited either by 400 μg/plate horseradish peroxidase (see Fig 2A) or 20U/plate catalase. Moreover, this activity could pass through dialysis membrane (Fig 2B). These results suggested that bacteriocin-like activity produced by viridans streptococci was H_2O_2. However, extremely large amounts of peroxidase and catalase were necessary for the complete inhibition than physiological amounts in saliva or expression on the cell surfaces of bacteria. Moreover, in contrary to our expectation, catalase activities on cell surface did not always inhibit bacteriocin-like activity of viridans streptococci. *P. aeruginosa,* MRSA, *Burkholderia cepacia, Enterobacter aerogenes* and *S. salivarius* had comparatively stronger catalase activities (359.2 ± 458mU/1×10^8cfu bacteria); however they were more susceptible to H_2O_2 than microorganisms with less strong catalase activities (average catalase activity of *S. oralis, S. mitis, S. sanguinis, E. coli, C. albicans and E. cloacae*: 46.0 ± 74.0mU).

Decomposition of H_2O_2 by bacterial cells in PBS and in saliva.

In PBS, MRSA catalase decomposed H_2O_2, whereas reagent H_2O_2 remained stable. Very small but detectable amounts of oxidants were produced during the spontaneous degradation of H_2O_2 in the presence of indigo carmine (which reacts with O_3, $^1O_2$, and H_2O_3); MRSA catalase inhibited this oxidant formation, in a dose-dependent manner. *Aspergillus niger* catalase also inhibited this oxidant formation. These data show that, in PBS, both *A. niger* catalase and MRSA catalase reduce the bactericidal potency of H_2O_2. However, in contrast to the oxidation-reaction outcome in PBS, H_2O_2 in saliva was decomposed by salivary peroxidase and more indigo carmine-reactive oxidant was produced than spontaneous decomposition in PBS (Fig 2C, line m0). Moreover, MRSA in saliva did not always inhibit the oxidant formation by salivary peroxidase (Fig 2C): at smaller doses of MRSA (line m1 and m2), MRSA catalase increased the oxidant-producing activity of salivary peroxidase (augmented bactericidal potency of H_2O_2); at larger doses of MRSA, this activity was inhibited (Fig 2C, line m3 and m4). For *A. niger* in saliva, similar results were obtained. *S. aureus* (catalase-negative strain TW4632) in saliva had no effect on the oxidant-producing activity of salivary peroxidase. To confirm the results, we studied a model reaction between *A. niger* catalase and horseradish peroxidase (HRP). We found that *A. niger* catalase interfered with the HRP activity (0.38ng/reaction mixture) in a similar manner (Fig 2D). Smallamounts of *A. niger* catalase augmented peroxidase activity as assessed by tetramethylbenzidine free base (TMB) oxidization reaction, whereas larger amounts of *A. niger* catalase inhibited TMB oxidation reaction, and thus inhibited peroxidase activity.

Aggregation and coaggregation.

Although H_2O_2 produced by viridans streptococci killed various microorganisms in vitro, it may not be strong enough to kill pathogens in vivo. Concentration or augmentation of the microbicidal activity of H_2O_2 in oral viridans streptococci is necessary for killing of pathogens.

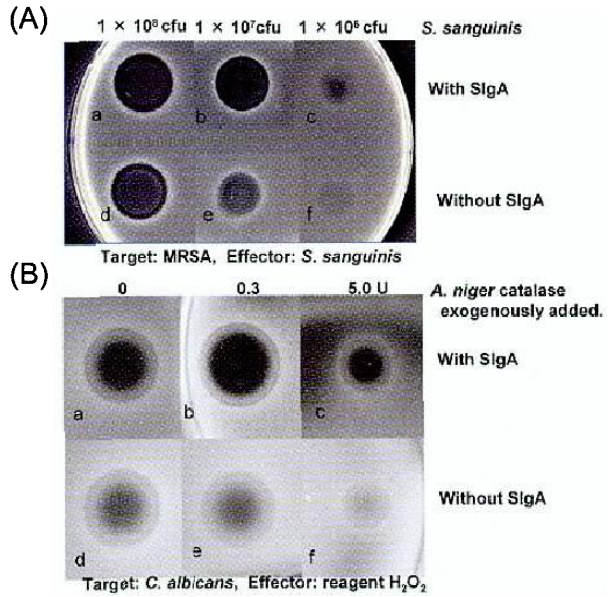

Figure 3.
Killing of MRSA by *S. sanguinis,* and killing of *C. albicans* by H_2O_2 in 50% saliva-agarose plate with or without exogenous colostral SIgA. Without exogenous SIgA, *S. sanguinis* (1×10^8 cfu and 1×10^7cfu) killed MRSA fixed in an agar plate in a dose-dependent manner (*A*-d, e), however, MRSA was not killed by 1×10^6cfu of S. sanguinis (*A*-f). The addition of 3.3 mg of colostral IgA, augmented the killing of MRSA, and larger areas remained clear around *S. sanguinis* at 1×10^8 cfu (*A*-a), 1×10^7cfu (*A*-b) and 1×10^6cfu (*A*-c). Very few C. albicans cells, even those just below the spot of reagent H_2O_2 (10 μl, 1%) (*B*-d, e, f), were killed in the absence of SIgA, even the presence of *A. niger* catalase. Addition of SIgA significantly augmented the killing (*B*-a, b, c). Large doses (5U) of *A. niger* catalase inhibited, however, small doses of catalase (0.3U) augmented the killing of *C. albicans.*

We could always observed bacterial aggregates in the oral cavity of healthy person (data not shown). Simple agitation of *Streptococcus sanguinis* for several minutes in colostral SIgA or saliva resulted in the formation of similar aggregations. Interestingly, IgA depletion almost completely inhibited the formation of aggregations, but not by IgG depletion (datanot shown). The results suggested the non-specific binding of salivary SIgA contributed to the aggregations of viri-

dans streptococci. In fact, colostral SIgA-coupled affinity column bound cell surface protein of *S. sanguinis,* fructose-bisphosphate aldolase. Simple agitation of viridans streptococci and *S. aureus* in saliva resulted in the formation of coaggregation. Flow cytometry indicated that there was SIgA antibody against *S. aureus* in both adult saliva and colostral SIgA (data not shown). Non-specific binding of SIgA to viridans streptococci and simultaneously specific binding to *S.aureus* may contribute to form coaggregation between the two bacteria.

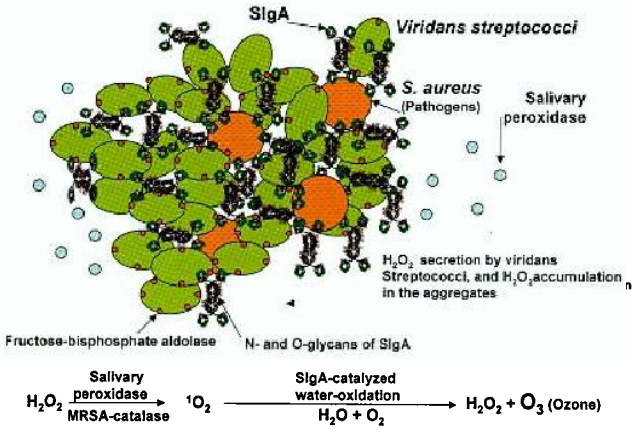

Figure 4.
The conclusion in diagram. Secretory IgA in saliva binds both viridans streptoscocci and MRSA (*C. albicans*) and formed microbial coaggregation. Small amounts of H_2O_2 produced by viridans streptococci was accumulated and concentrated in the coaggregates. H_2O_2 was converted by SIgA-catalyzed water-oxidation to ozone with potent microbicidal activity, and microbial catalase augments the ozone formation.

Assay of 4-vinyl benzoic acid oxidation.

Indigo carmine reacts with O_3, $^1O_2$, and H_2O_3. If $^1O_2$ is produced in the H_2O_2 degradation reaction, immunoglobulin-catalyzed water oxidation reaction to produce O_3 will be initiated in the presence of SIgA. Ozonolysis of 4-vinyl benzoic acid was measured by reversed-phase high-performance liquid chromatography. In the presence of SIgA and saliva, H_2O_2 oxidized 4-vinyl benzoic acid (RT, 11.47min) and produced 4-carboxybenzaldehyde (RT, 3.83min) and 4-oxiranyl-benzoic acid (RT, 4.31min), whereas neither H_2O_2 in PBS nor SIgA and saliva without H_2O_2 oxidized it; and 4-vinyl benzoic acid was similarly oxidized by ozone produced by UV irradiation. These data show that H_2O_2 is decomposed by saliva and is converted to ozone by the catalytic activity of SIgA.

Killing of MRSA by Streptococcus sanguinis and killing of C. albicans by H_2O_2, in a 50% saliva agarose-plate.

We examined whether SIgA augmented MRSA killing by *S. sanguinis,* or *C. albicans* killing by reagent H_2O_2 in the presence of saliva and exogenously added bacterial catalase. MRSA cultured on a 50% saliva-agarose plate produced confluent opaque colonies. Around the *S. sanguinis* colonies without exogenous SIgA, MRSA was killed by 1×10^8cfu of *S. sanguinis* (Fig 3A-d) and by 1×10^7cfu of *S. sanguinis* (3A-e), and the plate remained clear; however, MRSA was not killed by 1×10^6cfu of *S. sanguinis* (3A-f). The addition of 3.3 mg of colostral SIgA augmented the killing of MRSA, and larger areas remained clear around *S. sanguinis* at 1×10^8 cfu (3A-a, 1×10^7 cfu (3A-b) ($P<0.001$), and 1×10^6cfu (3A-c) ($P<0.004$). Figure 3B indicates *C. albicans* killing by reagent H_2O_2 in the presence or absence of SIgA, or *A. niger* catalase. Very few *C. albicans* cells, even those just below the spot of reagent H_2O_2 (10 μ l, 1%), were killed in the absence of SIgA (3B-d, e, f), even though contained saliva and *A. niger* catalase-and that the addition of SIgA significantly augmented the killing (3B-a, b, c). Large doses of *A. niger* catalase inhibited the killing of *C. albicans* (3B-c), even in the presence of SIgA, compared with the killing in cultures without this catalase (3B-a); however, large doses of this catalse did not completely eliminate production of $^1O_2$, because the augmentation in the killing of *C. albicans* in the presence of SIgA, compared with the killing in the absence of SIgA, indicate that $^1O_2$ is present; and small doses (0.3 U) of the catalase augmented the killing of *C. albicans* in the presence of SIgA (3B-b), compared with the level of killing in the absence of the catalase (3B-a) ($P<0.002$).

DISCUSSION

Uncontrollable spreading of MRSA in newborns in NICUs has been largely attributed to environmental risk factors (*14-16*). However, factors that involve the newborns themselves also seem to play an important role with regard to MRSA colonization. Colonization of normal flora may be one of these factors, for normal flora has the ability to inhibit colonization with pathogenic bacteria (*17-19*). The data in the present study demonstrate a previously unrecognized process that may help to maintain homeostasis in the oral cavity and thereby defend the oral environment against pathogens. Firstly, colonization of viridans streptococci in oral cavity, and secondly, coaggregation between viridans streptococci and pathogens, in conjunction with the catabolism of H_2O_2 in the aggregates to produce ozone by SIgA, seems to be the key event in this process. SIgA is responsible for mucosal defence, not only by immune exclusion (*20*), but also by direct killing of pathogens.

Interruption of the continuous flow of the colonization process at any point is likely to inhibit colonization with MRSA. Current methods of prevention of colonization, therefore, focus either on preventing patient-pathogen contact (*16,21,22*) or on preventing growth of colonizing microorganisms (*23,24*). However, none of the individual conventional control measures concerning these processes succeeded in controlling the spread of MRSA in an NICU (*15*). Moreover, mupirocin-resistant *S. aureus* (*25-26*) and vancomycin-

resistant *S. aureus* (*27*) have been reported. We indicate in the present study that none of the clinical characteristics of newborns interfere with the rate of MRSA colonization (*2*). Colonization with MRSA of a newborn's mucosa with no former bacterial occupants depends only on accidental contact with the bacteria and that, once contact is achieved, no newborn (irrespective of disease status) can avoid colonization. This phenomenon supports the conclusions of the study by Wickman (*28*), which showed that the bacterium that reaches the niche first will generate and prevail.

Beneficial and harmful effects of H_2O_2 produced by several bacteria because of alteration of human physiology have been the subject of special investigation. The α-hemolysin of viridans streptococci, a potential virulence factor, was identified as H_2O_2 (*29*), and streprolysin S and H_2O_2 synergistically injure vascular endothelial cells (*30*); however, H_2O_2 produced by lactobacilli may protect pregnant women from vaginosis (*31*). Ryan and Kleinberg (32) denied that H_2O_2 has an antagonistic effect on pathogenic bacteria, because H_2O_2 produced by viridans streptococci was rapidly degraded by bacteria in the oral cavity. However, we indicated in the present study that bacteria with high levels of catalase activity were not always highly resistant to H_2O_2. Catalase activity, with a few exceptions, tends to be inversely proportional to resistance. Exogenously added catalase protected MRSA from the antagonistic effects of H_2O_2, but the amounts required were extremely large compared with the amounts of catalase normally associated with MRSA cells (*3*).

SIgA contains various bacteria-binding sites in its glycans, in addition to its 4 Fab binding sites (*8,33*) and it binds to bacterial surface proteins, such as fructose bisphosphate aldolase of *Streptococcus pneumoniae* (*34*). In fact, the present study found that SIgA binds to viridans streptococci and forms aggregates. Possibly two mechanisms interfere with coaggregation formation between viridans streptococci and MRSA; 1) significant ζ potential (*35*) expressed on the MRSA's cell surface, 2) specific, but not non-specific binding of SIgA with MRSA. These two characteristics may have a role to construct the coaggregation composition in viridans streptococci excess, and may compensate for insufficient ability of H_2O_2 produced by one viridns streptococci cell.

Salivary peroxidase decomposes H_2O_2 into an indigo carmine-reactive oxidant, presumably either $^1O_2$, O_3, or H_2O_3 (*36,37*). However, this oxidant is not strong enough to kill some resistant microorganisms, such as *C. albicans* (*3*). In the present study, the addition of SIgA and small doses of *A. niger* catalase significantly augmented bactericidal and fungicidal effects, and more *C. albicans* cells were killed by the newly synthesized oxidant. Ozone may be produced by the SIgA-catalyzed water-oxidation reaction (*37*). If so, then the oxidant produced before the addition of SIgA is $^1O_2$, one of the starting materials of the reaction (*36*), and the products of 4-vinyl benzoic acid oxidation strongly suggest that ozone is present (*37*). In this situation, MRSA catalase may potentiate not the survival but, instead, the suicide of MRSA.

In conclusion, the results of the present study show that, in cooperation with H_2O_2-producing viridans streptococci and salivary components, SIgA can kill MRSA and *C. albicans*-and that this killing potential is augmented by bacterial catalase (Fig 7).

ACKNOWLEDGEMENTS

We gratefully thank Dr. Yoshiaki Suzuki for his critical reading of the manuscript.

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Current Situation and Prevention of HIV/AIDS

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ABSTRACT

The number of persons with HIV/AIDS is now about 40,000,000 in the world and is increasing especially in East/Southeast Asian countries. Although we often treat patients with HIV/AIDS, some medical/dental care providers in Japan still hesitate to treat them because of their lack of knowledge of HIV/AIDS. The basic outline of HIV/AIDS such as epidemiology, clinical course, transmission routes, and prevention from the infection is explained in this review. Furthermore, oral complications often seen in HIV infected patients are also presented and explained.

Key words: human immunodeficiency virus, viral load, saliva, oral complications

Epidemiology of HIV/AIDS

Since the establishment of the concept of acquired immune deficiency syndrome (AIDS) and discovery of human immunodeficiency virus (HIV) in the early 1980's, many aspects of this disease have been elucidated by the effort of many investigators around the world. However, a large number of persons have been infected with HIV and have suffered from the disease. The number of persons worldwide with HIV/-AIDS in December 2006 was estimated to be about 40,000,000 from the data of the World Health Organization (WHO) and the United Nations Programme on AIDS (UNAIDS). The increase in the number of patients in East/Southeast Asia is particularly conspicuous and is still rising, unlike the numbers in Europe/-North America which have plateaued. Although there are not so many patients in Japan compared with other East/Southeast Asian countries, patient numbers are increasing. Consequently, it has been suggested that the prevention program for HIV/AIDS in Japan has not been effective.
(*please insert Fig1&2)

Fig. 1
Number of people with HIV in 2006. This data is referred from "global summary or the AIDS epidemic December 2006" published by UNAIDS.

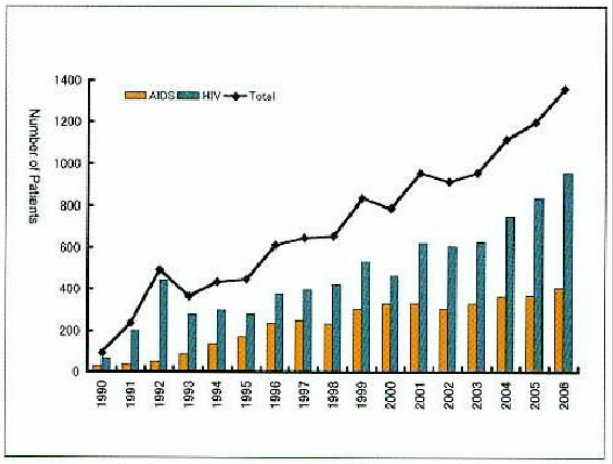

Fig. 2
Number of people newly infected with HIV in Japan. People diagnosed as AIDS are indicated as "AIDS" in this panel. On the other hand, those who have not suffered from AIDS definitive diseases are indicated as "HIV".

Natural history of HIV

HIV virions infect CD4 positive lymphocytes(CD4 cells). CD4 is a surface antigen presented on helper T cells or a part of macrophages and plays a role as the receptor of surface protein gp120 on HIV.
After the virions infect to CD4 cells and viral transmission occures, fever, oropharyngeal pain, general fatigue, lympho-adenopathy, rash, and sometimes diarrhea or nausea occurs 2-3 weeks later. These symptoms are called as "acute retroviral syndrome". Following this, an asymptomatic chronic HIV infection stage continues for several years. Immunodeficiency progresses with the decline in CD4 cell count. As a result, patients with HIV suffer from some opportunistic infections, so called "AIDS definitive diseases". This is a natural history of HIV infection in an aver-

age patient without antiretroviral therapy. However, the period from the time of HIV transmission to the stage of AIDS can be prolonged once patients receive antiretroviral therapy. We have already known the patients infected with HIV in the early 1980's, who have never suffered from opportunistic infections because of appropriate antiretroviral therapies.
(*please insert fig.3)

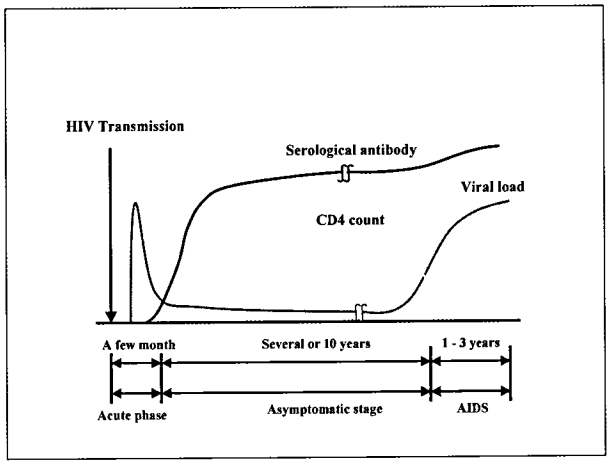

Fig. 3
Natural history of HIV infection in an average patient without antiretroviral therapies.

Laboratory tests for HIV

Serologic HIV antibody tests are effective and often utilized for diagnosis. The standard serologic test consists of screening tests (enzymelinked immunoassay: EIA or immunochromatography: IC) followed by a confirmatory Western blot (WB). However, false negative results are often due to testing in the window period. The time delay from infection to a positive screening test averages 10 to 14 days and that to a positive WB averages a few months. If the testee has acute retroviral syndrome, plasma HIV RNA PCR assay should be recommended because the window period is about half that of serologic antibody tests.
The specimens appropriate for detecting HIV infection are not only blood but body fluid such as saliva or urine. The saliva test is a device for collecting saliva and concentrating IgG for application of EIA tests for HIV antibody. It is a rapid test and it takes 20-30 minutes for measurement. The sensitivity and specificity of the test is 99.6% and 99.7% respectively. Potential advantages over standard serologic testing are the ease of collecting specimens, reduced cost, and better patient acceptance. However, it is somehow not prevalent and utilized at hospitals in Japan.
CD4 cell count in peripheral blood (CD4 count) is clinically an important test. It is a standard test to assess prognosis for progression to AIDS, to make therapeutic decisions regarding antiviral treatment and prophylaxis for opportunistic pathogens. In general, the risk of opportunistic infections becomes high and antiretroviral therapies should be started when baseline CD4 count is below 200/ul.

Routes for viral transmission

There are 3 main routes for viral transmission; blood, sexual contact and mother-to-child. This fact indicates that HIV transmission occurs via blood, semen, vaginal fluid and breast milk. Although HIV virions exist in the saliva of the patients, there have not been any reliable reports of viral transmission occurring via saliva. The number of virions in saliva is estimated to be less than found in other body fluids as mentioned above. In short, it appears that a particular concentration of virions is required for viral transmission and that the transmission rate can be minimized if the number of virions in the fluid is low. Skin is a strong physical barrier to HIV and the viral transmission does not occur if normal skin contacts body fluid containing virions.

Transmission by peripheral blood

Royce et al. reported the viral transmission rates according to the various routes. The transmission rate is more than 90% when blood of persons with HIV is transfused. However, as blood tests for various infectious diseases and rigorous blood-donor check systems are in force, the transmission rate by allogenic blood transfusion in Japan is below one in 6 million.
An occasion in which medical/dental care providers are exposed to HIV infected blood is a needle-stick accident. The transmission rate of that is estimated 0.1-1%. The transmission rate of needle-stick accident by injection needle is a little higher than that by surgical needle. Furthermore, quantity of exposed blood and viral load it contains also influence the transmission rate. In cases where the inside diameter of injection needle is large or viral load exposed is great, the risk of transmission becomes high.

Transmission by body fluid except for blood

The transmission rate by a sexual contact is 0.1-1%, the same as that by a needle-stick accident. Plasma viral load in HIV infected persons without antiretroviral therapies ranges from 10,000-1,000,000 copies /ml, and is same as or about 1 log higher than that in semen or vagina fluid. On the other hand, comparing the volumes exposed, that by semen or vagina fluid per sexual contact is, in general, about 1-2 log more than that by blood per needle-stick accident. Although there may be differences in the local immunity in the exposed area, the major determinant of the same transmission rate between sexual contact and needle-stick accident is total viral load exposed.

The risk of viral exposure in dentistry

Viral load in saliva is generally 2-4 log lower than that in blood or semen. It is suggested that the viral

transmission by saliva does not occur unless the exposed volume to mucosa is several hundred milliliters or liters at once. So, we can assume that there is no possibility of viral transmission by saliva or usual dental treatment. However, wearing gloves or goggles during dental treatment, use of disposable dental care devices or thorough sterilization may be needed as a standard precaution.

Some dental care providers in Japan are afraid of viral transmission from persons with HIV. They are concerned that persons with HIV may bite their fingers accidentally during the dental treatment. In this case, peripheral blood of person injured (which is without virions) may come into contact with the saliva of persons with HIV. However, it cannot be assumed that HIIV will be transmitted because viral load in saliva is very low. However, the situation changes somewhat if gum bleeding occurs or a dental operation is needed. In this case, there is high possibility of protection from viral transmission by adopting the standard precautions mentioned previously. This is because viral load in blood will be decreased by the use of gloves if needle-stick accidents occur or they are bitten accidentally.

There are several reports of viral transmission by needle-stick accidents. However, reports of viral transmission by dental treatments are rare.

Oral complications in HIV infected patients

(1) Candidiasis

Most common is pseudomenbraneous candidiasis, which is white painless plaques on the buccal or pharyngeal mucosa or tongue surface, typically in patients with HIV whose CD4 count is below 200/ul. Symptoms include diffuse retrosternal pain, dysphagia and odynophagia, which appear when esophageal candidiasis is complicated. Culture is the best method for speciation and sensitivity testing. Antifungal agents such as fluconazole or itraconazole are very effective and the treatment can be ceased in 7-14days.

(2) Aphthous ulcer

The cause is unknown in some cases with aphthous ulcer, although herpes simplex virus (HSV) or cytomegalovirus (CMV) often cause it. Use of antiviral agents is recommended if the cause is clearly demonstrated. Some of the unknown ulcers in patients whose CD4 counts are below 100 /ul repeatedly occur and become severe. In this case, it heals if immunity is recovered by antiretroviral therapies. Prednisone or thalidomide may be needed in refractory cases.

(3) Oral hairy leukoplakia

The cause is intense replication of Epstein Bar virus (EBV). It presents unilateral or bilateral adherent white/gray patches on lingual lateral margins of tongues and is not painful at all. It is almost is exclusively found in patients with HIV, whose CD4 counts are very low. It is improved by antiretroviral therapies.

(4) Gingivitis

HIV infected patients often have gingivitis; linear gingival erythema, necrotizing gingivitis, and necrotizing periodontitis. Patients with low CD4 counts tend to have severe cases. The pathogens are anaerobic bacterias and are the same as those causing gingivitis in patients without HIV. With antiretroviral therapies, the severe phase of gingivitis becomes rare. The standard treatment is routine dental care, occasionally curettage, debridement, or prescription of antibiotics for a short period.

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How Do We Choose the Daily Diet to Be Ingested?
-Behavioral Physiology of Feeding Behavior-

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ABSTRACT

How do we choose the daily diet to be ingested? For example, some kind of Japanese foods may not be acceptable for foreigners, and also, some kind of foreign cuisine may not be acceptable for the Japanese. This situation (to be cautious towards the new) is called "neophobia" in psychological term. It, consequently, defends the organism from danger elicited by the intake of unfamiliar food. When the poison is taken by mistake, the mechanism called conditioned taste aversion will be working. This mechanism is important with respect to refraining from taking the food with the same taste again. Recently, we found that the physical properties as well as the taste of food can work as conditioned stimulus for acquiring a conditioned food aversion. This paper is reviewing the behavioral mechanisms of food choice and food intake including the results of our recent study.

Key words: feeding behavior, food intake, neophobia, conditioned food aversion

INTRODUCTION

All animals, including human beings, are rendered to eat and drink everyday to assure the necessary nutrient intake. This behavior is called "feeding behavior". However, this behavior is not necessarily safe. For example, if the diet contains poison, the animal may become sick or die. To avoid such a situation, our feeding behavior has some important principles. This paper describes the principles for "How do we choose the daily diet to be ingested?".

Taste and Neophobia

Taste is the sensory system devoted primarily to a quality check of food to be ingested. It is known that there are five modalities of taste called "basic ta ste", such as sweet, salty, sour, bitter and umami. Sweet is always preferable for the majority of animals, because it is a signal of carbohydrates and energy. Sour and bitter are often unpreferable, because they are signals of putrification and usually poisonous alkaloids, respectively. Salty and umami are signals of minerals and L-amino acids. Suitable amount of these substances is necessary for our body, but overly amount of them may work as a poison. Therefore, we feel low concentration of them preferable, but high concentration of them unpreferable (Moskowits *et al.*, 1976; Yamaguchi, 1998). This situation derives from the following principle: "We may eat anything which we feel preferable."

However, this principle is often not perfect. To avoid unexpected food poisoning, we have a second principle called "neophobia". Neophobia is a psychological term for that we feel danger for the unfamiliar food and consequently avoid its intake. For example, we know many foreigners hesitating to eat some kinds of Japanese foods. If we take only familiar foods, the possibility of becoming poisoned by food may decrease.

Conditioned Taste Aversion

Usually the swallowed food improves body conditions and it elicits an (often highly) preferable feeling. However, food may occasionally cause toxic effects. Animals can remember of the chemical/chemosensory feature (i.e. taste) corresponding to a particular food that once elicited internal malaise after being ingested, and they will develop an aversion for it. This behavior is called conditioned food (taste) aversion (CTA), and it is the 3rd principle of our feeding behavior.

Figure 1 shows the typical result of a CTA experiment demonstrated by Dr. Yamamoto and his collaborators (Yamamoto *et al.*, 1995). When the rats are injected by i.p LiCl, which elicits internal malaise as an unconditioned stimulus (US), after drinking of saccharin (conditioned stimulus; CS), they keep decreasing the intake volume of the originally preferred saccharin. On the other hand, the rats injected by physiological saline instead of LiCl increase the intake volume of saccharine day by day, because saccharin has sweet and preferable taste for rats.

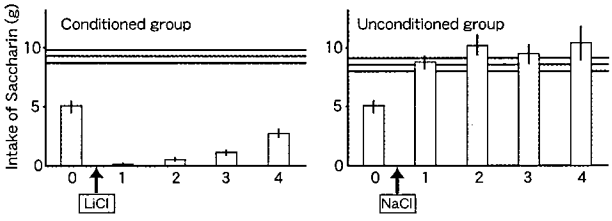

Fig 1.
A typical conditioned taste aversion. Rats of conditioned group reduced their intake of saccharin (left), whereas those of the unconditioned group did not show aversion to saccharin, even if they had neophobia (right).

The behavioral mechanism and its phenomenological aspects have been reported by many researchers. Today, it is known that some regions of brain, such as the parabrachial nucleus (PBN), amygdala and the insular cortex, play critical roles to acquire the CTA (Yasoshima *et al.*, 2006; Yamamoto *et al.*, 1994, Bures *et al.*, 1998 etc.).

Conditioned Food Aversion Elicited by the Hardness of Food

The daily foods which we eat are not simple taste solutions. They have physical properties, such as texture and temperature, as well as chemical properties which elicit taste sensation to the animals. Although some researchers have reported the temperature of drinking water working as CS for conditioned food aversion in behavioral experiments (Nachman, 1970; Heth, 1985), very little is known about whether the physical properties of food can work as CS for leading to conditioned food aversion. Therefore, we designed some behavioral experiments to answer this question.

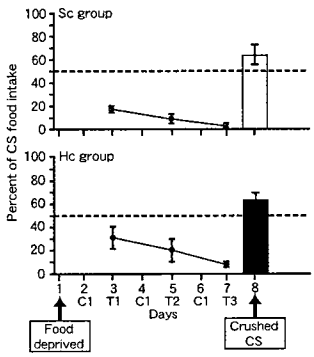

Fig 2.
Percent of CS pellet intake for Soft pellet conditioned group (Sc; upper) and Hard pellet conditioned group (Hc; lower). Columns show percentage of crushed CS pellet intake for each group. C1-C3, conditioning days; T1-T2, test days.

At first, we examined whether the hardness of food could work as CS (Sako *et al.*, 2002). Wistar rats were divided into two groups: a hard pellet conditioned (Hc) and a soft pellet conditioned group (Sc). Each rat of the Hc and Sc group was given access to hard pellet (H; 79.3±13.0 gf/mm^2) and soft pellet (S; 37.0±4.2 gf/mm^2), respectively, and they were given an ip injection of LiCl (resulting in the malaise) as the US. The pellets contained the same ingredients. After this conditioning, a two-dish preference test, one dish filled with H and another dish filled with S, was carried out for 24 h on the 3rd, 5th and 7th post-injection days. Re-conditioning was performed every 2nd days. On the 8th day, crushed H and S in powder form were presented instead of S and H pellets. Figure 2 shows the preference percent (= 100 x consumed volume of CS / sum of consumed total volume). As a result, the rats of each group have avoided CS pellets. Because they did not avoid the crashed CS, it is clear that this aversion learning was elicited by the hardness of pellets as the CS. Our study suggests that the hardness of food as well as the taste can work as a CS for the conditioned food aversion.

Conditioned Food Aversion Elicited by the Temperature of Drinking Water

We also designed behavioral studies to examine whether the temperature of drinking water can work as a CS (Sako *et al.*, 2004). In the first experiment, the rats were divided into a cold distilled water (dw) group (Cd) and a warm dw group. The trained rats of the Cd group were subjected to aversive conditioning to 5℃ dw, and the rats of the Wd group were subjected to aversive conditioning to 40℃ dw by ip injection of LiCl. Their mean licking suppression rate (MLS) (%) ={1-(licks for 10 s in experimental group)/(licks 10 s in control group)} x 100 was measured. Rats in the control group were injected by physiological saline instead of LiCl. Figure 3 shows the result. The rats of both groups could acquire the conditioned temperature aversion for CS temperature. But they did not generalize to any taste stimuli with CS temperature. Figure 4 shows the result when the rats were subjected to aversive conditioned to 5℃ sucrose (Cs group) or 40℃ sucrose. In this situation, the rats of both groups generalized to all sucrose solution, even if they had different temperature from the CS solution. These results suggest the following two facts. (1) The rats have an ability to acquire conditioned food aversion elicited by the temperature of drinking water as the CS. (2) The taste gives priority over the temperature to form conditioned food aversion.

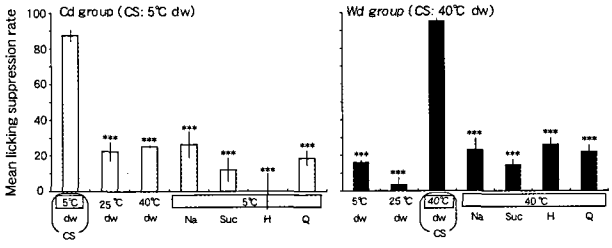

Fig 3.
Mean licking suppression rate for cold distilled water conditioned group (Cd; left) and warm distilled water conditioned group (Wd; right). dw: distilled water, Na: NaCl, Suc: sucrose, H: HCl, Q: quinine-HCl.

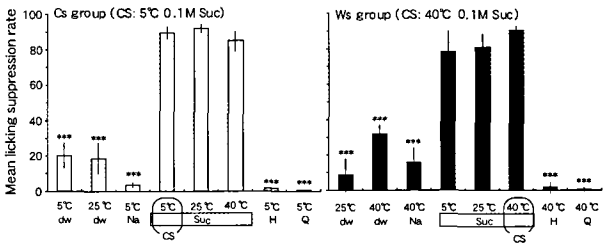

Fig 4.
Mean licking suppression rate for cold sucrose conditioned group (Cs; left) and warm sucrose conditioned group (Ws; right). Abbreviations are the same as in Fig. 3.

This paper briefly reviewed some organizing principles for the feeding behavior. We should remember of that our safe life is greatly approved by these principles.

ACKNOWLEDGEMENTS

The Author is grateful to Drs. Takashi Yamamoto (COE, Grad, Sch. Dent., Osaka Univ., Osaka, Japan) and Zoltan Karadi (Pecs Univ. Med Sch., Hungary) for their support in study. I also thank staffs of Dept. Oral Physiol., Asahi Univ. Sch. Dent. and Dept. Behav. Physiol., Grad. Sch. Human Sci., Osaka Univ.

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Tool-Using Monkey Brains Possess Latent Evolutionary Precursor of Language

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Bimodal neurons in the monkey intraparietal cortex, integrating visual and somatosensory informations, code the image of the self-body, which is subject to intentional modification. When trained to use a tool, it becomes an extension of the hand both physically and perceptually, resulting in alteration of the body image in accordance with the characteristics of the tool at hand. In above bimodal neurons, use-dependent expansion of the receptive field occurred only when the monkeys held a tool and intended to use it as an extension of their hand. This would constitute the neural correlate for modification of the body schema as a basis of assimilation of the tool into our own body. PET imaging studies confirmed that this cortical area is active when monkeys using the rake. Also, we found that these neurons can code the body-image projected onto the video monitor, perhaps corresponding to its iconic representation.

During the course of above training, behavioral analyses suggested that a novel mode of somatosensory-visual integration seemed to develop in order to organize adequate bodily movement to manipulate the tool, possibly subserved by reformation of the neural circuitry in which molecular genetic processes in the cortical area described above are involved. Indeed, augmented expression of messenger RNA of neurotrophic factors associated with learning was induced in the corresponding cortical region only during the training to use the tool, but not after monkeys acquired the skill. Corresponding to this period, emergence of novel cortico-cortical projections between temporo-parietal junction and the intra-parietal cortex were detected in monkeys that were trained to use tools, therefore, enabling to integrate the tool in their own body image by presence of a self-objectification mechanism.

When above described representations were further advanced, it would become totally free from physical constraints of the actual world to become a symbolic one to represent evolutionary precursors of higher cognitive functions, and might eventually lead to evolution of human language or to the metaphysical thoughts.

Role of Childhood Trauma in the Neurobiology of Depression and Stress Vulnerability

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INTRODUCTION

The rapid globalization of economy and culture, and the progress of information technology have increased the prevalence of psychiatric disorders such as depression and anxiety in all generation from child to elderly population. It has been a serious public health problem of adults in Japan that the number of suicide victims has been over 30,000, approximately 5 times of traffic accident victims in the last decade. On the other hand, in children, the number of child abuse including physical and sexual abuse has been increasing. Epidemiologic studies indicate that childhood traumatic experiences such as child abuse or maternal separation are at increased risk for the development of depression. Persistent sensitization of central nervous system (CNS) circuits as a consequence of early life traumatic stress, which are integrally involved in the regulation of stress and emotion, may represent the underlying biological substrate of an increased vulnerability to stress.

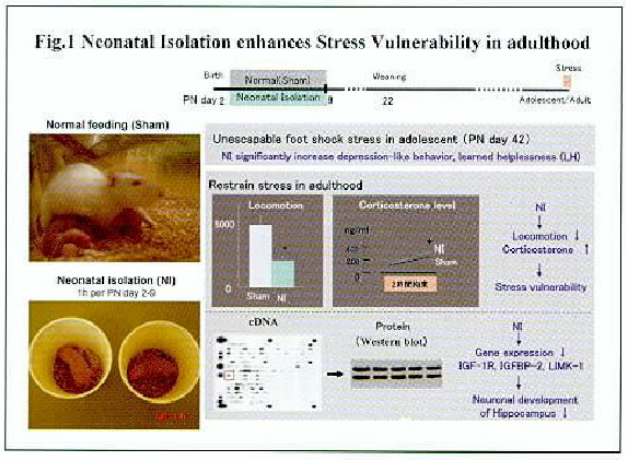
In this conference, the authors demonstrate current research including our study on the role of childhood trauma in the neurobiology of depression and vulnerability to stress.

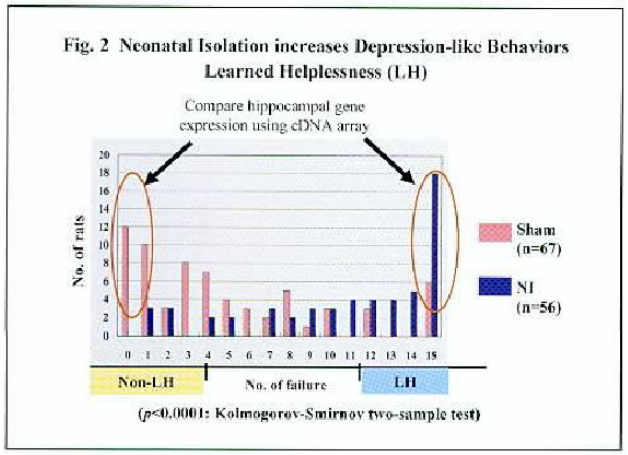
DEPRESSION AND HIPPOCAMPUS

Advances in neuroimaging technology such as magnetic resonance imaging (MRI) allow the examination of subtle changes in both regional structure and function that are associated with the pathophysiology of depression. The neuroanatomical volumetric MRI studies indicate the decreases in hippocampal volume among depressive patients compared with control. Hippocampus plays important role in emotional regulation as well as memory. This hippocampal atrophy is suggested to be underlying the pathophysiology of depression and/or vulnerability to stress.

NEONATAL ISOLATION AND STRESS VULNERABILITY

The authors investigated the influence of neonatal isolation (NI) on the stress vulnerability and depression-like behavior (learned helplessness: LH). As shown in Fig. 1, in NI group, pups were isolated from the mother and siblings, and placed in individual cages for 1h per day on post-neonatal (PN) day 2-9. In non-NI group, the mothers and pups were left together until weaning (PN day 21). On PN day 42 (adolescence), the prevalence of depression-like behaviors (LH) in NI group was significantly higher than that in non-NI group, which confirmed that NI was at increased risk for the development of depression in rats as well as in human (Fig.2). On PN day 90 (adulthood), the corticosterone level during restrain stress in NI group was significantly higher than that in non-NI group, which suggested that NI induced an endocrinological stress vulnerability. When NI group rats were handling and feeding under enrich environment, the prevalence of LH behavior has decreased (Fig.3).





NEONATAL ISOLATION AND HIPPOCAMPAL GENE EXPRESSION

Hippocampus was isolated and used for DNA microarray, real-time PCR, immunohistochemistry and Western blot analysis. In LH experiment on PN day 42, we found that significant lower expression of LIM domain kinase 1 (LIMK-1) mRNA in NI rat hippocampus, which is reported to play an important role in actin-related spine and synapse formation. In restrain stress experiment on PN day 90, we also found that significant lower expression of insulin-growth factor-1 receptor (IGF-1R) and IGF binding protein-2 (IGFBP-2) mRNA, which are known to be neuronal growth factors.

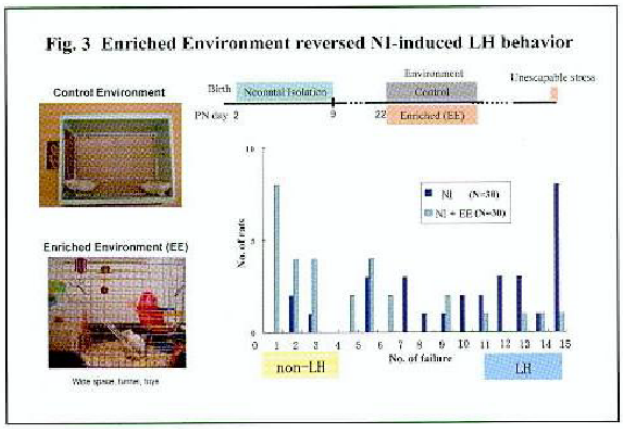


Fig. 3 Enriched Environment reversed NI-induced LH behavior

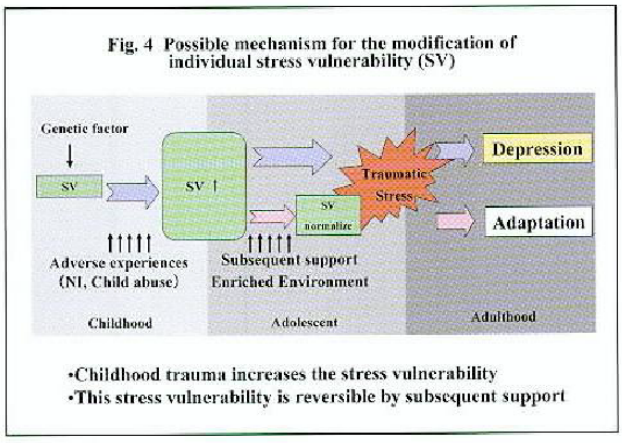


Fig. 4 Possible mechanism for the modification of individual stress vulnerability (SV)

CONCLUSION

These above our findings provide the evidence that childhood trauma like NI in rat induced the vulnerability to stress and increase a risk of depression behaviorally and endocrinologically in adolescence and adulthood (Fig. 4). In NI rat hippocampus, the expression of several growth factor-related genes and spine formation-related genes were decreased, which suggest NI-induced stress vulnerability might be mediated by genetic modification. This NI-induced stress vulnerability was reversible by Environment Enrichment. These results suggest that early adverse experiences should be addressed in the clinical care of children, adolescents and adults with psychiatric disorders. It should be noted, however, that it is generally difficult to infer from animal studies on the effects of childhood stress on human development.

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Developmental Origins of Adult Health and Disease: A Pediatric Perspective in Current Japan

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ABSTRACT

The origins of adult disease are considered to relate with fetal undernutrition, and this concept is termed "developmental origins of adult health and disease" (DOHaD). We describe here epidemiological data of Japanese children and adolescents and discuss whether DOHaD can be confirmed or not in current Japan. It was found that systolic blood pressure of 3-year-old healthy children was inversely correlated to birth weight, and positively correlated to weight at 3 years. Hyperinsulinemia, blood pressure, transaminase and prevalence of metabolic syndrome in obese children and adolescents were inversely correlated to birth weight, and positively correlated to current weight and waist circumference. DOHaD is thus considered to be a concept that applies to Japanese children and adolescents. The essential mechanisms of "DOHaD" are considered to be two mismatches. One is a mismatch of growth and development with environmental influences, especially nutrition; the other is a mismatch of the pre- versus the postnatal environment. As epigenetic adaptation concerning the mismatches will increase the risk factors for adult disease, we consider that adult disease will be increased by the aforementioned mismatches in current Japan. Pediatrician and school teachers should therefore understand the concept of DOHaD and educate children and their families regarding appropriate food intake for the prevention from adult disease promptly.

Key words: Japanese children and adolescents, obesity, mismatch concept, epigenetic mechanisms, health education

INTRODUCTION

The prevalence of obesity in Japanese children and adolescents has increased from 5% to more than 10% over the last two decades. This amounts to a serious public health problem, since childhood obesity, which is the leading cause of hyperinsulimenia and insulin resistance, is associated with adult disease, such as hypertension (HT), dyslipidemia, type 2 diabetes mellitus (DM) and metabolic syndrome (MS) in adulthood. These diseases are caused by both genetic factors and environmental factors after birth. Environmental factors after birth, the so-called "lifestyle factors", are more closely related with the development of these diseases than genetic factors, as indicated by concordance with the recent rapid increase in fat consumption in Japan. An adequately healthy lifestyle avoiding obesity from childhood will prevent certain adult diseases, and thus pediatricians of today must be concerned with health education of children and adolescents in order to prevent obesity.

In 2004, Barker and colleagues proposed the concept of "developmental origins of adult health and d isease" (DOHaD), which considers intrauterine growth restriction (IUGR) to be associated with increased risks for MS and its related disease in later life (1). The supply of nutrients to the fetus is the major influence that determines its growth, and this supply depends on the mother's body composition and size, her nutrient store, what she eats during pregnancy, and the transport of nutrients to the placenta and transfer across it. DOHaD suggests that health education for children and adolescents is important for preventing them from bearing offspring that will be at risk for MS and its related diseases, because maintenance of a healthy lifestyle for mothers is necessary for preventing IUGR. We briefly review the epidemiological data of Japanese children and adolescents, and discuss DOHaD can be confirmed or not in current Japan. If this hypothesis is confirmed, the need for health education for children and adolescents will be emphasized.

DOHaD in healthy Japanese children

We previously reported relationships between birth weight, current body weight and blood pressure (BP) in 195 3-year-old healthy Japanese children (2).

Systolic blood pressure (SBP) was inversely correlated to weight at birth, and positively correlated to weight at 3 years. Children whose birth weight was greater than 3520 g had a mean SBP at 3 years of 3.0 mmHg below that of those whose birth weight was 2999 g or less. Mean SBP at 3 years for children whose weight at 3 years exceeded 16.8 kg was 9.4 mmHg higher than that of age-matched children whose weight was 14.2 kg or less (Table 1). We found an increase of 0.12 mmHg in the children's SBP with each increment of 1 mmHg in the SBP of their mothers. The SBP at 3 years in children of mothers who had had pretibial edema during pregnancy (101.0±8.8 mmHg) was significantly higher compared with children whose mothers did not have edema (96.6±9.6mmHg).

DOHaD in obese Japanese children and adolescents

We also reported the relationships between birth weight, current visceral fat accumulation to hyperinsulinemia and insulin resistance in 967 obese Japanese children and adolescents (650 boys and 317 girls; age range 6-15 years) (3). We divided the subjects into four groups according to their birth weight or SD score, and compared anthropometric measurements, maximum preperitoneal fat thickness of the abdominal wall (Pmax), BP, serum insulin levels, homeostasis model assessment-insulin resistance (HOMA-R) and quantitative insulin sensitivity check index (QUICKI) among the quartiles. The fasting serum insulin levels

Table 1. Mean systolic blood pressure (mmHg) according to weight at birth and at 3 years after being divided into four groups of b

	Weght at 3 years (kg)				
Weight at birth (g)	~14.2	14.3~15.3	15.4~16.7	16.8~	Mean±SD (n)
~2999	95.7 (17)	97.2 (15)	100.4 (7)	105.4 (8)	98.5±8.0 (47)
3000~3179	95.9 (14)	91.7 (11)	101.9 (19)	102.6 (5)	98.0±10.7 (49)
3180~3519	91.2 (13)	95.8 (10)	93.7 (13)	106.0 (15)	97.0±10.9 (51)
3520~	88.0 (6)	95.2 (14)	94.2 (11)	99.4 (17)	95.5±9.2 (48)
Mean±SD (n)	93.6±8.8 (50)	95.1±9.1 (50)	97.9±9.2 (50)	103.0±9.5 (45)	

Reference date from [2]

Table 2. Characteristics of 650 obese boys after being divided into four groups according to birth weight.

	group A (n=162)	group B (n=163)	group C (n=162)	group D (n=163)	ANOVA *p*
Range of birth weight (g)	1740 - 3005	3006 - 3250	3254 - 3505	3508 - 4875	-
Birth weight SD score	-0.55 ± 0.76¶†*	+0.14 ± 0.33†*	+0.64 ± 0.33*	+1.63 ± 0.64	<0.0001
Gestational week	38.3 ± 1.5¶†*	39.2 ± 1.1†*	39.5 ± 1.0*	39.8 ± 1.2	<0.0001
Age (year)	10.2 ± 2.4	10.4 ± 2.2	10.6 ± 1.9	10.4 ± 1.9	NS
Height (cm)	141.3 ± 14.2*	142.3 ± 12.4*	143.9 ± 12.9	145.4 ± 12.8	<0.05
Weight (kg)	53.9 ± 15.9*	54.9 ± 14.7*	58.1 ± 17.3	58.6 ± 16.0	<0.05
Height SD score	+0.73 ± 1.07*	+0.79 ± 1.15*	+0.87 ± 1.09*	+1.31 ± 0.96	<0.0001
Percent relative weight (%)	+50.8 ± 12.2†	+51.5 ± 11.3†	+54.2 ± 15.7	+51.9 ± 13.2	NS
Waist circumferences (cm)	84.0 ± 10.4†*	84.3 ± 9.4†	86.3 ± 11.1	86.5 ± 9.6	<0.05
Hip circumferences (cm)	87.7 ± 9.2†*	88.1 ± 8.4†*	90.4 ± 10.1	90.3 ± 8.9	<0.01
Pmax (mm)	11.1 ± 3.2¶	11.8 ± 3.4	11.3 ± 3.4	11.2 ± 3.5	NS
SBP (mmHg)	116 ± 14	116 ± 12	115 ± 13	115 ± 12	NS
DBP (mmHg)	58 ± 9	58 ± 8	57 ± 9	56 ± 7	NS
Serum insulin (ng/ml)	20.2 ± 19.5†*	17.4 ± 10.4	16.5 ± 11.4	15.8 ± 9.1	<0.05
HOMA-R	4.3 ± 3.9†*	3.8 ± 2.3	3.7 ± 2.6	3.4 ± 2.0	<0.05
QUICKI	0.32 ± 0.03	0.32 ± 0.03	0.33 ± 0.03	0.33 ± 0.03	NS

¶: $p<0.05$ vs the value of group B, †: $p<0.05$ vs the value of group C, *: $p<0.05$ vs the value of group D using Bonferroni/Dunn's *post hoc* test; NS, not statistically significant.

Reference date from [3]

Table 3. Stepwise multiple regression analysis of MS defining factors and metabolic markers validating the involvement of current height and weight, birth weight and gender (n=126)

		r	F	
Waist circumference	Height	·0.427	33.0	
	Weight	1.177	250.1	R^2=0.755
	Birth Weight	NO		p<0.0001
	Gender	NO		
SBP	Height	NO		
	Weight	0.447	29.7	R^2=0.198
	Birth Weight	·0.237	8.3	p<0.0001
	Gender	NO		
DBP	Height	NO		
	Weight	0.195	5.3	R^2=0.143
	Birth Weight	·0.388	20.9	p<0.0001
	Gender	NO		
HDL	Height	NO		
	Weight	NO		
	Birth Weight	NO		
	Gender	NO		
TG	Height	NO		
	Weight	NO		
	Birth Weight	NO		
	Gender	NO		
FBG	Height	NO		
	Weight	NO		
	Birth Weight	NO		
	Gender	NO		
GPT	Height	·0.396	7.9	
	Weight	0.595	18.0	R^2=0.149
	Birth Weight	·0.219	6.4	p<0.0001
	Gender	·0.202	5.7	
HbA1c	Height	NO		
	Weight	0.264	9.3	R^2=0.062
	Birth Weight	NO		p<0.005
	Gender	NO		
Serum insulin	Height	NO		
	Weight	0.502	43.7	R^2=0.313
	Birth Weight	·0.396	27.2	p<0.0001
	Gender	NO		

SBP, systolic blood pressure; DBP, diastolic blood pressure; GOT, glutamate-oxaloacetate transaminase level; GPT, glutamic-pyruvic transaminase level; T-Chol, total cholesterol level; HDL, high-density lipoprotein cholesterol level; LDL, low-density lipoprotein cholesterol level; TG, triglyceride level; FBG, fasting blood glucose level; HbA1c, hemoglobin A1c. NO, not obtained

Waist circumference, HDL, TG, GPT and serum insulin levels were log-transformed before analysis.
Reference date from [4]

Table 4. Prevalence of metabolic syndrome in 261 Japanese obese boys according to birth weight and waist circumference after being divided into 3 groups

	Birth weight (g)			
waist circumference (cm)	1740~3120	3130~3425	3430~4875	Total
64.0~82.0	4 (10.8%)	0 (0.0%)	1(3.8%)	5 (5.6%)
	37	*27*	*26*	*90*
82.5~88.5	7(29.2%)	3 (10.3%)	6 (218.2%)	16 (18.6%)
	24	*29*	*33*	*86*
89.0~116.0	9 (33.3%)	8(26.7%)	5 (17.9%)	22 (25.9%)
	27	*30*	*28*	*85*
Total	20 (22.7%)	11(12.8%)	12 (13.8%)	43 (16.5%)
	88	*86*	*87*	*261*

Numbers in italics represent total number of each group

and HOMA-R were highest in the quartile with the lowest birth weight or SD score (Table 2). The birth weight and SD score were inversely related to the serum insulin levels and HOMA-R, positively related to QUICKI, after adjustment for Pmax. These findings suggest that both the intrauterine environment and current visceral fat accumulation are related to hyperinsulinemia and insulin resistance, and the subsequent development of MS in obese Japanese children.

Moreover, we have reported on the relationships between current weight-to-birth weight ratio [WBWR] and blood pressure, transaminase and hyperinsulinemia in 126 obese Japanese children and adolescents (97 boys and 29 girls; age range 9-12 years) (4). The subjects were divided into the MS group or Non-MS group using criteria proposed for Japanese children. There were no significant differences in age or anthropometric measurements between the two groups. However, birth weight in the MS group was lower than that in the Non-MS group, while WBWR of the MS group was higher than that in the Non-MS group. Blood pressure, serum insulin and glutamic pyruvic transaminase levels correlated positively with WBWR (Table 3). We divided 261 obese boys into nine groups according to lower, middle, heavier birth weight and thin, middle, wider waist circumference. The highest prevalence of MS was among those with lower birth weight and wider waist circumference (Table 4). Given these findings, DOHaD is thus considered to be a concept that applies to Japanese children and adolescents.

Mechanisms of DOHaD

Fetal growth is constrained by the nutrients and oxygen provided by the mother. A poor intrauterine environment, such as that in a mother with malnutrition, a smoking habit and/or inappropriate dieting, will result in fetal growth retardation. When the maternal-placental nutrient supply fails to match the fetal nutrient demand, the fetus adapts to poor nutrition by changing its metabolism, altering its secretion of hormones and the sensitivity of tissues to them, redistributing its blood flow to protect key organs, especially the brain, and slowing its growth rate. This adaptation, called "programming", is beneficial to fetal survival. However, this programming will permanently alter the structure and function of the tissues and body of the newborn.

The downstream effects of poor fetal nutrition include insulin resistance in muscle, liver and adipose tissue, poor development of pancreatic s-cell mass and function, decreased replication of kidney cells, and reduced activity of placental 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 2. This programming is considered to be the individual's adaptation for the mismatch of nutrition with growth and development during the fetal stage. Moreover, the second mismatch of the pre- versus the postnatal environment will contribute to increasing the risk factors for adult disease. The underlying mechanisms of this programming involve epigenetic modifications in nonimprinted genes induced by aspects of the developmental environment, which modify gene expression without altering the DNA sequence.

A pediatric perspective for DOHaD in current Japan

In Japan, the average birth weight has been decreasing to under 3000 g and the incidence of low birth weight infants has been increasing up to 9.5% in 2005. Maternal nutrition intake is insufficient since the average energy intake is around 1700-1880 kcal per day without increasing during pregnancy. Such a poor nutritional condition, which is also associated with a more western lifestyle, is expected to result in a trend of increased risk for adult diseases in current Japanese children. Pediatrician and school teachers should therefore understand the concept of DOHaD and educate children and their families regarding appropriate food intake for the prevention from adult disease promptly.

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Over-Nutrition, Inflammatory Periodontal Disease, and Metabolic Syndrome

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ABSTRACT

It has been indicated that severe periodontal disease is not merely a local infectious disease but low-grade inflammatory condition for the host. The prevalence of over-nutrition appears to increase rapidly in both Western and Asian countries. Both obesity and type 2 diabetes, whose pathophysiology is strongly associated with over-nutrition, act as risk factors for periodontal disease. Periodontal disease, in turn, appears to suppress insulin action known as insulin resistance and to cause persistent low-grade inflammatory reaction, thereby exhibiting additive inflammatory effect to the adipose-derived inflammatory condition in subjects with metabolic syndrome, which ultimately accelerate various metabolic disorders such as diabetes and atherosclerosis. Therefore, it is essential to understand the molecular basis of these associations for the development of effective preventive strategies against these unwanted side effects. The purpose of this study is to 1) summarize the mechanisms by which over-nutrition acts as a risk factor for periodontal disease, and to 2) provide most recent understandings as to how local periodontal infection evokes systemic inflammatory responses.

Key words; over-nutrition, diabetes, obesity, adipocytokine, inflammation

INTRODUCTION

The prevalence of over-nutrition appears to increase rapidly in both Western and Asian countries. It is estimated that there are now as many obese people in the world as there are people suffering from hunger (Campbell, 2000). Obesity is associated with many metabolic disorders such as impaired glucose tolerance, hypertension, and dyslipidemia, clusters of which is known as metabolic syndrome characterized by far increased risk of developing coronary heart diseases.

Periodontal disease, the most prevalent dental disease in the adults, has been considered as a local infectious disease caused by the infection of gram-negative anaerobic periodontal bacteria and recognized as the leading cause of tooth loss in the adults. Both obesity and type 2 diabetes, develop as a result of over-nutritious status, act as risk factors for periodontal disease (Saito *et al.*, 1998; Nelson *et al.*, 1990). In addition to that, several observations in different laboratories have suggested that periodontal disease inversely influences the patho-physiology of metabolic syndrome via several distinct pathways (Nishimura *et al.*, 2007). Periodontal disease has been suggested to suppress insulin action in diabetic subjects, thereby accelerates the fatigue of insulin secreting pancreatic b-cells (Iwamoto *et al.*, 2001). Periodontal disease has also been suggested to promote the development of atherosclerosis (Beck *et al.*, 2001). As the number of the subjects with metabolic syndrome is still estimated to increase dramatically (Campbell, 2000), it is essential to understand the molecular basis of these associations for the establishment of effective disease marker for better diagnosis and treatment and for the development of effective preventive strategies against these unwanted associations.

Therefore, in this review, we first summarize the mechanisms by which over-nutrition acts as a risk factor for periodontal disease. We then provide most recent understandings as to how local periodontal infection evokes systemic inflammatory responses for the host.

Mechanisms by which diabetes and obesity act as a risk factor for periodontal disease

It is well-known that diabetic subjects are susceptible to severe periodontitis. This association was firmly established by the observation of Pima Indians who develops type 2 diabetes with highest incidence in the world (Nelson *et al.*, 1990). As for type 1 diabetes, since the prevalence of this disease is relatively higher in Northern Europe, periodontal condition of juvenile subjects with type 1 diabetes was compared with that of systemically healthy controls (Löe, 1994). The results indicated that juvenile subjects with type 1 diabetes exhibited far increased attachment loss as compared with control subjects, and the authors who conducted this study proposed to consider periodontal

disease as the sixth complication of diabetes mellitus (L?e, 1994). However, regardless the diabetic condition, obesity has also been recognized as another important risk factor for periodontal disease (Saito *et al.*, 1998).

Then, the question arises as to how both obesity and diabetes influences periodontal pathology. Obese subjects are characterized by abdominal accumulation of mature adipose tissues. Adipose tissue is known to produce many kinds of biologically active molecules known as adipocytokines (Matsuzawa, 2006). Many of adipocytokines are essentially the same as so-called pro-inflammatory cytokines such as tumor necrosis factor-a (TNF- α), interleukin-6 (IL-6) (Matsuzawa, 2006). In fact, circulating TNF- α , IL-6 value as well as c-reactive protein (CRP), which is produced by hepatocytes in response to IL-6, are all increased in obese subjects and decline with weight loss (Nishimura *et al.*, 2007). Thus, we can easily speculate that these inflammatory adipocytokines further up-regulate periodontal inflammation, results in the increased periodontal tissue destruction. However, as documented, the subjects with type 1 diabetes, who are usually lean diabetic subjects, also develop periodontal disease easily, suggesting that hyperglycemia itself has some roles in periodontal pathology. To investigate this issue, we compared cytokine productivity and the activation of associated signal transduction molecules in bacterial endotoxin-stimulated macrophages pre-exposed to either high glucose or normal glucose conditions. The results indicated that LPS-stimulated macrophages pre-exposed to high glucose produced higher amounts of TNF- α and MCP-1 as compared with the cells cultured in normal glucose (Iwata *et al.*, 2007). Interestingly, the cells produced higher amounts of TN F- α and MCP-1 cultured in hyperglycemic condition are characterized by increased phosphorylation of c-Jun N-terminal kinase (JNK), and both high JNK activity and high cytokine production appeared to be strongly associated, as specific inhibitor for JNK dramatically suppressed these cytokine production (Iwata *et al.*, 2007). Therefore, it can be concluded that, in diabetic conditions, high inflammatory cytokine productivity from macrophages possibly induced by high JNK activity may also accelerate periodontal tissue destruction when exposed to bacterial antigens such as endotoxin. Our current hypothesis indicating the high susceptibility to periodontal tissue destruction in both obese and diabetic cases is summarized in Figure 1.

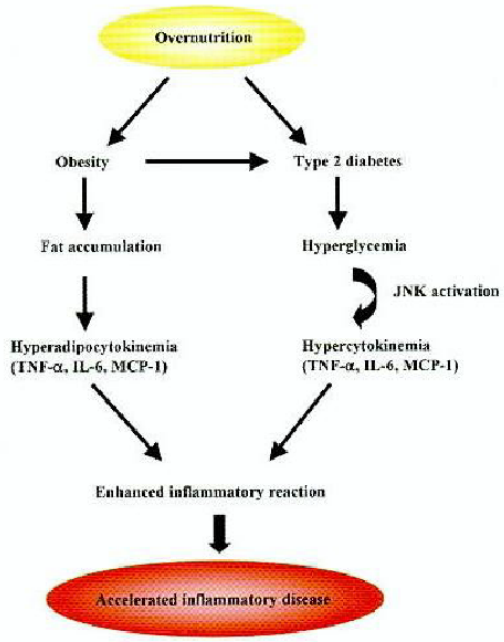

Figure 1.
Hypothetical scheme of the mechanisms by which overnutrition accelerates inflammatory tissue destruction.
Overnutrition is a risk factor for both obesity and diabetes. Obesity is also a strong indicator of type 2 diabetes. In obese subjects, fat accumulation results in the enhanced production of adipocytokines, which exacerbates inflammatory reactions, thereby promoting inflammatory tissue destruction. In contrast, in diabetic subjects, hyperglycemia is induced. High glucose induces high cytokine production in macrophages via enhanced JNK activation. These cytokines also exacerbate inflammatory reactions as in obese subjects.

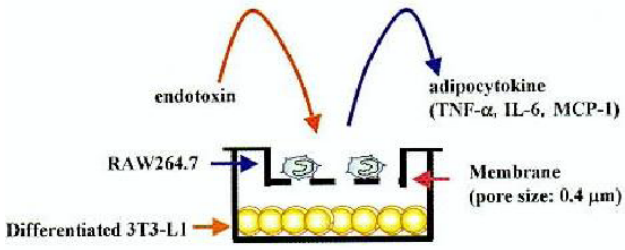

Figure 2.
Co-culture system of adipocytes and macrophages.
3T3-L1 pre-adipocytes were differentiated into mature adipocytes in a lower chamber of trans well cell culture system. RAW264.7 cells, murine macrophage cell line, were cultured in an upper chamber of trans well culture system. Both upper and lower chambers were separated by a membrane with 0.4 mm pore size, which only allows soluble factors to penetrate the membrane.

Mechanisms by which local periodontal infection cause persistent low-grade inflammatory reaction in the body

There is increasing evidences supporting the concept that severe periodontal disease is not merely a local infectious disease but low-grade inflammatory condition for the host (Nishimura F *et al.*, 2007). For example, CRP value is elevated in some severe periodontitis subjects when measured by highly sensitive assay and declines with successful therapy (Iwamoto *et al.*, 2003). CRP is generally believed to be synthesized in the liver in response to inflammatory stimuli such as IL-6 stimulation (Gabay and Kushner, 1999). Therefore, in severe periodontitis subjects, at least inflammatory stimuli enough to produce such level of CRP should be evoked in the liver. Additionally, as severe periodontal inflammation is associated with increased insulin resistance (Iwamoto *et al.*, 2001), at least such insulin resistance-causing inflammation must be maintained either in liver or in adipose tissues, as both hepatocytes and adipocytes are insulin sensitive cell types incorporating blood glucose in response to insulin stimuli.

Recently, two studies investigating the gene profiling of adipose tissue indicated that mature adipose tissues contained substantial amounts of the genes normally expressed in activated macrophages (Weisberg *et al.*, 2003; Xu *et al.*, 2003). These authors, in fact, demonstrated macrophages infiltration by histochemical techniques. Additionally, they suggested that infiltrated macrophages, unlike tissue resident Kupfer cells in the liver, were originally migrated through circulation. Furthermore, both macrophages and adipocytes express toll-like receptor-4, a receptor for bacterial endotoxin (Shi *et al.*, 2006). TLR-4 was recently found to act as a receptor for saturated fatty acid such as free fatty acid as well, and thus, further exacerbated inflammatory reaction is s evoked in mature adipose tissues (Shi *et al.*, 2006). Based on these studies, we hypothesized that activated macrophages by periodontal antigens could migrated into adipose tissue, interacting with adipocytes, thereby producing higher amounts of adipocytokines. To test this hypothesis, we established co-culture system between adipocytes and macrophages and stimulated these cells with bacterial endotoxin (Figure 2). The results indicated that co-culturing of these cells resulted in the marked increase in IL-6 and MCP-1 production when stimulated with endotoxin as compared with the productivity by each cell culture (Yamashita *et al.*, 2007). Therefore, we speculate that activated macrophages initially stimulated by periodontal antigens are easily recruited into the adipose tissues, interacting with resident mature adipocytes, and these cells produce much higher amounts of IL-6 and MCP-1. MCP-1 could further recruit circulating macrophages (Kanda *et al.*, 2006), while IL-6 could be transferred into the liver via portal vein, stimulate hepatocytes to produce CRP (Gabay and Kushner, 1999) (Figure 3). Thus, adipose tissue may act as an inflammation-amplifying organ for the body.

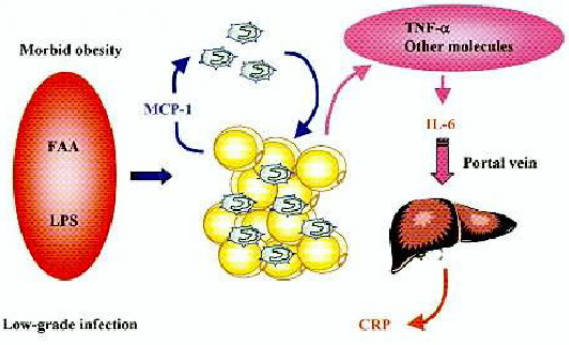

Figure 3.
Hypothetical scheme of the mechanisms by which adipose tissue accelerates inflammatory reactions.
In markedly obese subjects, saturated fatty acid such as free fatty acid (FFA) is induced by lipolysis. In contrast, in low-grade inflammatory situation such as periodontal disease, bacterial endotoxin (lipopolysaccharide: LPS) is released into the circulation. Both FFA and LPS act as ligands for TLR-4, exacerbating macrophage-adipocyte interaction, which over produces inflammatory cytokines such as MCP-1 and IL-6. MCP-1 further promotes macrophage infiltration into adipose tissues, while IL-6 stimulates hepatocytes to produce inflammatory marker such as CRP via portal vein.

CONCLUSION

Up-dated knowledge of the molecular basis for close association between periodontal disease and metabolic syndrome is provided. Further detailed investigation such as gene profiling of this adipocyte-macrophage interaction will be an attractive subject to explain further detailed molecular basis of this important association.

ACKNOWLEDGMENTS

This work was supported by a Grant-in Aid (17 390562) from Japan Society for the Promotion of Science.

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Oral Administration of Liposomal Lactoferrin Inhibits LPS-Induced Alveolar Bone Destruction in Rats

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ABSTRACT

Lactoferrin (LF) having immuno-suppressive effects may be a candidate of a preventive or therapeutic modality of periodontitis. The aim of this study is to examine inhibitory effects of bovine LF (bLF) on alveolar bone destruction caused by lipopolysaccharide (LPS). The bLF inhibited up-regulation of TNF-α and RANKL mRNA expression and eliminated down-regulation of OPG mRNA expression in ST2 cells with LPS-stimulation. The bLF treatment of co-cultures of osteoblasts and bone marrow cells dose-dependently suppressed osteoclasts formation induced by LPS. For animal study, liposomal bLF (L-bLF) was used to promote the absorbability of bLF from the intestinal mucosa. Wistar strain rats divided into three groups with 7-day-preadministration of L-bLF, non-liposomal bLF (NL-bLF) and vehicle (Control) in drinking water, respectively. Orally administrated L-bLF inhibited increase of osteoclast and reduced up-regulation of TN F-α immunoexpression caused by LPS. It is suggested that oral administration of L-bLF may be a useful preventive or therapeutic modality of periodontitis.

Key words: liposomal lactoferrin, oral administration, LPS, osteoclast, TNF-α

INTRODUCTION

Superficial periodontal tissues are constantly exposed to plaque-associated bacteria and bacterial lipopolysaccharide (LPS), which can induce an inflammatory reaction and the consequent tissue destruction. Cytokines, which are rapidly synthesized and secreted from host cells by LPS stimulation, appear to play an important role in periodontal tissue destruction. It has been reported that increased levels of cytokines such as tumor necrosis factor (TNF) -α, interleukin (IL)-1 β, and IL-6, were detected in inflamed gingival tissue and gingival crevicular fluid at inflamed site. (Okada et al. 1988)

Previously, we have used an experimental model in which initial periodontal tissue destruction is provoked by topical LPS-application, including infiltration of polymorphonuclear leukocytes (PMNLs) and exudative macrophage, vascular dilatation and edema in the sub-junctional epithelium, destruction of collagen fibril and induction of osteoclast. Up-regulation of T NF-α, IL-1-b (Miyauchi et al., 2001) and CXC-chemokine (Miyauchi et al., 2005) is evident in the periodontal tissue and is responsible for tissue destruction. T-herefore, we speculated that appropriate regulation of cytokine production by using natural immunomodulator might be useful in reduction of periodontal tissue destruction.

Lactoferrin (LF) is an 80-kD iron-binding glycoprotein of the transferrin family with a wide spectrum of biological activities, can modulate the inflammatory response by binding potentially toxic-free iron. In addition, LF may affect immunological functions by modulating cytokine production of TNF-α, IL-1-b, and IL-6 (Legrand, 2005). Although the mechanism by which LF modulates cytokine production is not well understood, previous work has shown that oral (Hayashiba et al., 2004) or local (Guillen et al., 2000) administration of bovine LF (bLF) actually inhibited inflammation in rat arthritis. It is, therefore, possible that oral administration of bLF may be preventive or therapeutic treatment for periodontitis. The bLF has been focused on as the food origin material with high safety. However orally administrated bLF is fragmented by gastric pepsin or trypsin in the stomach, so we improved bLF's robustness to gastric digestion by liposomalization. (Ishikado et al., 2005). Liposomes prepared from naturally occurring biogradable and non-toxic lipids have been proposed as an efficient carrier for local delivery of therapeutic agents. Some studies have already demonstrated that liposomal bLF (L-bLF) can enhance anti-inflammatory effects of bLF (Tif et al., 2001, Ishikado et al., 2004).

The aim of this study was to examine the inhibitory effect of oral administration of L-bLF on periodontal tissue responses caused by LPS stimulation *in vitro* and *in vivo* experimental studies.

MATERIALS AND METHODS

Reagents

bLF was purchased from Morinaga Milk Industry Co., Ltd. (Japan). L-bLF was prepared according to the previously described method (Ishikado et al., 2005). Actinobacillus Actinomycetemcomitans (Aa-LPS) were kindly provided by Professor Tatsuji Nishihara (Kyushu Dental College).

RNA Extraction and RT-PCR Analysis

ST2 (a bone-marrow-derived osteogenic cell line) cells (1 x 10^5cells/dish) were seeded and cultured in a-MEM containing 10% FBS for 2 days. At 0, 2, 4, 6, 12 and 24 hrs after incubation with Aa-LPS (100 ng/mL), total RNA was extracted and cDNAs were synthesized from 1 mg of total RNA with Rever Tra Ase (TOYOBO CO., LTD., Osaka, Japan). Aliquots of total cDNA were amplified with specific primer pairs and KOD-Plus-DNA Polymerase (TOYOBO CO.) using a MyCyclerTM thermal cycler (BIO-RAD, Tokyo, Japan).

Osteoclast Formation Assay

Primary osteoblasts were obtained from calvariae of newborn ddY mice and Bone marrow cells (BMC) were collected from femora and tibiae of 6-week-old male mice. Primary osteoblasts (1 x 10^4 cells) and BMC (2 x 10^5 cells) were co-cultured for 5 days in α-MEM containing 10% FBS in 96-well tissue culture plates. Co-cultures were incubated in the presence of Aa-LPS (1 μ g/ml) for the final 3 days. Some co-cultures were pretreated with or without bLF (1 or 10 μ g/ml). Then co-cultures were stained for tartrate-resistant acid phosphatase (TRAP).

Experimental protocol

The experimental protocol was approved by the animal care committee of Hiroshima University.

A total of 126, 7-week-old (about 215 g), Wistar strain male rats were randomly divided into three groups of 42 each and treated with 10.0 g/L liposomal bLF including 2.125 g/L bLF (L-bLF group), 2.125 g/L non-liposomal bLF (NL-bLF group) and vehicle (Control group) in drinking water, respectively. On day 7 of bLF treatment, LPS application was done. A cotton roll (2 mm in diameter and 1 cm in length) saturated with 5 mg/ml LPS from E. *coli* (Sigma Chemical, St. Louis, Mo., USA) was placed on the palatal marginal periodontal tissues in the right and left molar regions for 1 hr. The cotton roll was changed every 20 min. Six rats in each were sacrificed at 0, 1, or 3 hrs, or 1, 2, 3, or 7 days after the LPS treatment.

Measurement of serum cytokines level

At sacrifice, blood plasma was collected for cytokine assay. Assessment of the concentrations of nine cytokines was performed using a BioRad Bio-Plex cytokine assay (BioRad Laboratories Inc., Hercules, CA, USA).

Tissue preparation

The right and left upper molar regions were resected en bloc, fixed in a periodic-lysine paraformaldehyde solution for 24 hrs at 4℃ and cut into two slices, which included the first or second molars, respectively, at the buccopalatal plane parallel to each distopalatal root. They were then decalcified in a 10% ethylenediaminetetraacetate solution for 10 days at 4℃. The serial paraffin sections were cut in the direction parallel to the long axis of the tooth, including root apex and stained with hematoxylin and eosin (HE) for histological examination.

Immunohistochemistry

To investigate the expression of TNF-α, in the periodontal tissues, immunohistochemical staining was performed using the high polymer (HISTOFINE simple stain, NICHIREI, INC., Tokyo) method. The goat polyclonal anti-TNF-α antibody (Santa Cruz Biotechnology, Inc., CA) was diluted 1:100 in PBS. The color was developed with 3-3'-diamino bentizine tetrahydrochloride.

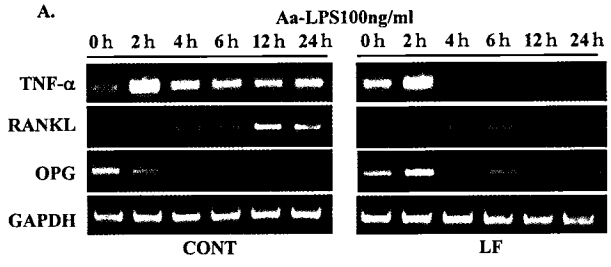
Histometric analysis

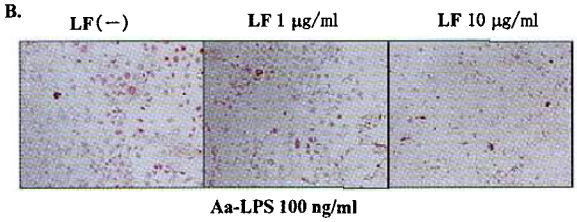
More than 12 representative TRAP stained specimens from each experimental group were selected. The number of TRAP positive cells appeared along the alveolar bone margin was counted with 1 mm height from the alveolar crest. TRAP-positive cells with two or more nuclei were defined as osteoclasts while mononuclear cells were defined as preosteoclasts. The mean ± SD values were determined. We used a Scheffe's test to check for differences between each experimental group in each time. Probabilities of less than 0.05 were considered to be significant.

RESULTS

bLF inhibits osteoblast-mediated osteoclastogenesis induced by LPS

LPS stimulated TNF-α and RANKL mRNA expression in ST2 cells while OPG mRNA expression was down-regulated. Treatment with bLF suppressed the LPS-induced up-regulation of TNF-α and RANKL mRNA and eliminated down-regulation of OPG mRNA by LPS (Fig.1A). Aa-LPS (100 ng/mL) stimulated TRAP-positive cells formation in co-culture system of ST2 cells and BMC. The bLF dose-dependently suppressed the number of TRAP-positive cells induced by LPS (Fig.1B).





Figre. 1.
(A) Effect of bLF on the expression of TNF-α, RANKL and OPG mRNA in ST2 cells with LPS stimulation. ST2 cells are treated with Aa-LPS (100ng/mL) for 0-24 hrs. The bLF inhibits up-regulation of TNF-α and RANKL mRNA expression and eliminates down-regulation of OPG mRNA expression by LPS-stimulation. (B) Effect of bLF on LPS induced osteoblasts mediated osteoclastogenesis in a co-culture system of primary osteoblasts and bone marrow cells. The bLF treatment of co-cultures of osteoblasts and bone marrow cells dose-dependently suppresses osteoclasts formation

Orally administrated L-bLF reduces the number of osteoclast appeared along alveolar bone margin after topical application of LPS.

In LPS-untreated animals with 7 day-administration of each drinking water, only a few osteoclasts were seen along the alveolar bone surface (Control; Fig.2Aa, L-bLF; Fig.2Ad). In the control group, LPS application caused marked increase of osteoclasts at 3 hrs and at 3 days and the alveolar bone surface of the periodontal ligament (PDL) side showed an irregular shape due to bone resorption (Fig.2Ab). And then, the number of osteoclasts decreased and returned to the normal range by day 7. On the contrast, in L-bLF groups, increase of osteoclasts was not evident through the experimental period. (Fig.2Ae). In NL-bLF, number of osteoclasts was also decreased but some specimen from NL-bLF group showed induction of osteoclasts.
To identify osteoclasts and pre-osteoclasts we performed TRAP staining on the serial sections. At 3 hrs and 3 days after LPS treatment, large numbers of TRAP-positive osteoclasts and pre-osteoclasts were found in the control group (Fig.2Ac). Meanwhile, in the two bLF groups, the numbers of TRAP-positive cells were almost constant during experimental period. (Fig.2Af).
Fig.2B shows number of TRAP-positive osteoclasts (a) and preosteoclasts (b). In the untreated control, the numbers of TRAP-positive osteoclasts and pre-osteoclasts in the counting area were 5.5 ± 1.0 and 8.3 ± 0.8, respectively. LPS application caused biphasic increase of osteoclast (Fig.2Ba) and pre-osteoclasts (Fig. 2Bb) peaking 3 hrs ($p<0.05$) and 3 days ($p<0.01$) comparing to the untreated animals. Meanwhile, in the two bLF groups, the numbers of TRAP-positive osteoclasts and pre-osteoclasts were almost constant throughout the experiment period. Especially at 3 days, the numbers of TRAP-positive osteoclasts and pre-osteoclasts in the L-bLF group were significantly ($p<0.01$) smaller than those in control group.

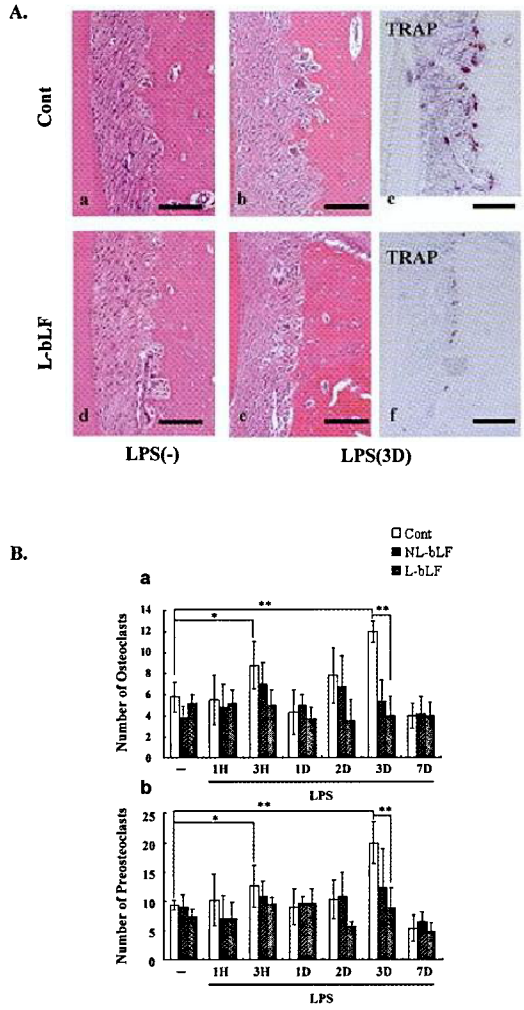


Fig. 2.
(A) Effects of liposomal bovine LF (L-bLF) on alveolar bone destruction caused by LPS application in the periodontal tissue. In the control, numerous osteoclasts are seen along alveolar bone margin at 3 days after LPS-application (b, c). On the contrast, in both bLF groups, bLF treatment suppresses the osteoclast induction caused by LPS application. Particularly the inhibition in L-bLF group is prominent (e, f). Osteoclasts and preosteoclasts are positively stained for TRAP. (c, f) a, b, d, e) HE staining, c, f) TRAP staining scale bars: 100 μm
(B) Effects of bLF on number of TRAP positive cells appeared along alveolar bone surface. a); TRAP-positive osteoclasts, b); TRAP-positive pre-osteoclasts. Data are mean ± SD. *$p<0.05$, **$p<0.01$

Orally administrated L-bLF reduced immunoexpression of TNF-α in periodontal tissue after topical application of LPS

Fig.3 showed the immunohistochemical staining of TNF-α at 3hrs after LPS application. Many epithelial cells were strongly positive for TNF-α. Especially in JE, the superficial layer was consistently positive for TNF-α. (Fig.3a). Numerous TNF-α-positive cells were also observed in PDL (Fig.3b). At 3 days after TNF-α-positive cells were decreased but still presented. The L-bLF group showed small number of TNF-α lpha-positive cells in JE (Fig.3c), no positive reaction for

TNF-α (Fig.3d) was seen in PDL. At 3 days, no TNF-α-positive cells were detected in periodontal tissue in L-bLF group.

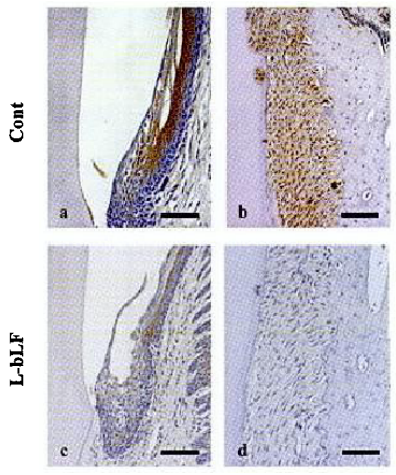


Figure. 3.
Effects of liposomal bovine LF (L-bLF) on immunoexpression of TNF-α in the gingival tissue and periodontal ligament (PDL). In the control, TNF-a-positive cells are observed in junctional epithelium (a) and PDL cells (b) at 3 hrs after LPS-application. On the contrast, in the L-bLF, TNF-α positive cells can not observe in periodontal tissue (c, d). scale bars: 100μm

Concentration of pro-inflammatory cytokine in serum

Serum levels of TNF-α, IL-1β and IL-6 showed a tendency to increase 1 hr after LPS stimulation. However there is no significant difference in all experimental groups compared to untreated control (data not shown).

DISCUSSION

In the present study, we demonstrated that bLF eliminated LPS effects on osteoblast; up-regulation of TNF-α and RANKL expression and down-regulation of OPG expression. In fact, bLF reduced TRAP positive osteoclast formation in an osteoblast/bone marrow cell co-culture system induced with LPS stimulation. It is suggested that bLF may inhibit osteoblast-mediated osteoclastogenesis by regulating the expression of TNF-α, RANKL and OPG in osteoblast. Lorget et al. (2002) also reported that bLF inhibited osteoclastogenesis and reduced bone resorption using a rabbit mixed bone cell culture. It is known that the down-regulation of TNF-α by bLF can be partially related to the LPS binding properties of bLF and the inhibition of LPS binding to CD14 by bLF via competition with LPS binding protein (Legrand, 2005). Recently, other mechanism for inhibition of cytokine production was reported by bLF. The bLF may down-regulate the LPS-induced TNF-α production via the inhibition of binding of NF-κB to the TNF-α promoter following internalization of bLF in monocytic cells (Harversen, 2002). However, the mechanism by which bLF modulates cytokine production is not well understood. It is also needed to study direct effect of bLF on osteoclasts.

Oral administration of both form of bLF inhibited increase of osteoclasts after application of LPS. This is the first study to show therapeutic effect of bLF on LPS-induced periodontitis. Moreover, administration of bLF suppressed immunoexpression of TNF-α in periodontal tissue but not influenced its serum level. These findings suggest that bLF might reduce osteoclastogenesis through suppressing local level of TNF-α in periodontal tissue after topical application of LPS.

TNF-α, which can cause production of other pro-inflammatory cytokines and stimulation of bone resorption is one of key cytokines involved in pathogenesis of periodontitis. In this animal model, TNF-α down-regulation induced by bLF is a critical causative event for inhibition of osteoclastogenesis.

In the present study, we had decided to use L-bLF to protect bLF from enzymatic digestion in stomach. Ishikado et al (2004) recently reported that liposomalization of bLF improved bLF's robustness to the gastric digestion and proposed that oral administration of L-bLF could be a novel active constituent useful for preventive and therapeutic treatment of inflammatory disease. Expectedly, oral administration of L-bLF exhibited more stable and effective suppression than did NL-bLF in increase of osteoclasts and pre-osteoclasts in this animal model.

The transport routs through which L-bLF orally administrated was delivered to periodontal tissue remained unclear. It is reported that bLF entered into the blood circulation via the lymphatic pathway after oral administration of bLF (Takeuchi, 2003). L-bLF transported through blood circulation system may locally affect cells existing in periodontal tissue. In addition, L-bLF in drinking water also can directly penetrate into periodontal tissue through JE and act on it's receptors on tissue resident cells. Moreover it is reported participation of the intestinal tract immunity system of the mesentery lymph node and the Payer's patch (Harada, 2002). The further studies are needed to clarify this issue.

Recently, Singh et al (2004) reported that iron sequestration by bLF inhibited to bacterial biofilm formation. L-bLF can be used for prevention of periodontitis thorough inhibition of plaque formation. Moreover, accumulating evidence indicates not only that systemic diseases affected periodontal condition but also that periodontitis is a potential risk factor for increased morbidity or mortality for several systemic diseases including cardiovascular diseases, pregnancy complications and diabetes mellitus (Moutsopoulos, 2006). In particular, it is well documented that severity of chronic periodontitis is positively associated with plasma TNF-α levels in type 2 diabetic patients (Engebrestson, 2007). Genco et al. (2005) also demonstrated that TNF-α produced from adipocytes is a pathogenic factor linking obesity to diabetes and periodontal disease. These findings are indicating that

systemic approach for periodontal therapy and prevention are needed in addition to the local control of infection and inflammation. Therefore, we suggest that orally administrated L-bLF may be more effective in the prevention of periodontitis especially in patients with diabetes.

CONCLUSION

Oral administration of L-bLF significantly reduced alveolar bone resorption through suppression of TNF-α locally produced from host cells with LPS stimulation. Moreover bLF may directly osteoblast mediated osteoclastogenesis. The results indicate the possibility of the therapeutic usage of bLF for LPS-induced periodontitis. However there is a possibility that L-bLF in the diet may stimulate transient systemic and intestinal antibody responses. We should carefully consider the oral administration for therapeutic and/or preventive usage of food protein such as bLF.

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Education session

Innovation of Education for Oral Health Science

Challenges and Opportunities in Dental Education: A Perspective from Hong Kong

University of Hong Kong, Professor

Lakshman Samaranayake

Dental Education in the United States: Responding to Challenges

University of Washington, Professor

Martha J. Somerman

Dental Education in Malaysia

University of Malaya, Professor

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Challenges and Opportunities in Dental Education: A Perspective from Hong Kong

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ABSTRACT

It is clear that the challenges facing dental education are very similar worldwide. These challenges will not be adequately addressed with anything less than a concerted response from the government, community, alumni, and industry. Only then can the Faculties or Schools of Dentistry flourish and provide a stimulating and quality environment for graduates to develop their capabilities and to hone their competitive advantage for a challenging career in a fast-changing world. In this article, taking Hong Kong as an example, the author addresses the multifarious challenges in education, research, and resource support that constantly challenges the delivery of dental pedagogy worldwide.

INTRODUCTION

Dental education is the fountain from which all dentistry flows. It is the framework on which scientific findings and advancements in oral care are discovered and implemented. A strong dental education system produces a strong dental profession, resulting in the highest level of oral health care for the public.

In Hong Kong, there is only a single institution that provides undergraduate dental education: the Prince Philip Dental Hospital, where the Faculty of Dentistry of The University of Hong Kong is based. Over a period of 25 years, this health care facility has helped mould generations of dentists, giving them the knowledge, skills in science and critical thinking, and ethical principles necessary to meet most of Hong Kong's oral health needs. The Faculty of Dentistry also provides a world-renowned postgraduate programme, which has grown from strength to strength, with a current enrolment of more than 140 postgraduates from 14 countries worldwide. This student diversity is not surprising, as our Faculty is considered the top facility for the delivery of postgraduate dental education in China, and possibly in the whole of Asia.

Furthermore, the Faculty of Dentistry has contributed to research that has led to innovative treatments, new technologies, and better ways of delivering oral health care. News of such advancements is regularly shared with others — internationally in peer-reviewed science journals, and locally through the Faculty's continuing education programmes, which enable local practising dentists and specialists to stay up-to-date with the latest scientific discoveries in materials, instruments, and techniques.

Since their establishment, the Dental Faculty and Hospital have functioned as an important safety net for dental treatment for some of the underserved people in Hong Kong. The Faculty is particularly fortunate to have a nucleus of specialists who accept referrals and serve as a key resource for the practising dental community.

In fact, the Faculty of Dentistry is celebrating its Silver Jubilee this year, marking 25 years of serving the people of Hong Kong and the region through excellence in learning, discovery, patient care, and community engagement. Yet, dental education in Hong Kong faces many challenges. Challenges that are common to all dental faculties worldwide and in this presentation I shall deal with these in turn, in terms of education, research, and infrastructure support.

Dental Education

The Cost of Dental Education

The strong association between dental education and improved public oral health is well known. But in order for dental education to continue to play its vital role, it is important to ensure a strong supply of qualified, diverse dental students and faculty who have access to state-of-the-art teaching and research facilities.

Owing to the one-on-one teaching and very close supervision necessary, dental education is among the most costly professional training programmes worldwide (1). This is especially so in Hong Kong, where dental treatment at the Faculty is virtually free for teaching patients, so the costs of both education and treatment cannot be offset by dental charges. While costs continue to grow, government support has gradually declined over the years. Although dentistry is

one of the top three sought-after programmes by undergraduates both from Hong Kong and abroad our student enrolment cannot be increased as a result of many factors, including political considerations. Yet, private dental schools are mushrooming all over the world and represent sound financial enterprises. One way of ameliorating the public financial burden of educating dentists would be to open a full-fee-charging dental school for foreign students in Hong Kong, and use some of the revenue generated for local education and research in dentistry. This initiative could also help talented students from lower-income families or those with financial hardships to pursue a dental career.

The Learning Environment

In line with modern educational philosophy, the Faculty of Dentistry launched a new approach to undergraduate dental education some 8 years ago. The pedagogy of open-discovery problem-based learning (P-BL) was introduced in July 1999 to develop the personal, intellectual, and leadership qualities of our students. Ours is only one of three dental schools in the world, the others being the University of Southern California School of Dentistry and the Univerity of Malmo, Sweden where this open-discovery or the pure mode of PBL education is fully practiced (2). Here, students study a large series of integrated problems from year 1, devoid of didactic lectures. The learning is competency driven, self-directed, problem-based, and group-based. During PBL, students are encouraged to express themselves effectively and reflect on critical issues as well as on their role in society. This programme characterises the transformation from a teaching to a learning environment, where active student-student interaction is seen as one of the cornerstones of the learning process.

The proponents of the pure PBL tend to disagree with other variants of PBL that have proliferated over the years. These so called `hybrid` or guided-discovery PBL programmes are thought to be less resource intensive than the pure PBL programmes. In reality though analysis of both modes of teaching have shown that neither programme is more or, less resource intensive than the other.

Dental Research

Working together, dental educators and scientists at the Faculty and industry partners both from Hong Kong and abroad have made the Faculty quickly evolve into perhaps the finest centre for dental research in Asia. Creating an environment where research can flourish and grow has been no mean achievement for an institution that began life purely as a teaching centre for undergraduates two and a half decades ago.

In the 21st century, dental schools must have the capacity to conduct collaborative research across many disciplines, and they must be full and equal participants in medical research programmes. Dental academics, in alliance with industry and research grant councils (eg the National Institutes of Health in the United States), have developed new and innovative products and equipment, such as fluorides and implants. Research has also led to advances in orthodontics and orofacial reconstruction that improve a patient's self-esteem and daily functioning. Dental schools provide the greatest potential to turn clinical findings into practical applications through their connections with students, practising dentists, industry, community clinics, and public-policy advocates. In addition, dental schools not only serve as a source of new approaches and technologies, but they also share their new knowledge with others by way of updated curricula and continuing education courses.

If inadequate resources are provided for dental research, then it is highly likely that advances looming on the horizon might never come to fruition. Imagine the impact on the public's oral and general health if dental researchers developed a vaccine to prevent periodontal (gum) diseases or discovered gene therapies to prevent oral cancers. Furthermore if dental schools were to survive within a university environment it is imperative that they pursue quality research that compete with various other research programmes in other allied faculties. The research that is so pursued should be aligned to Nuffield principles of scholarship of discovery, scholarship of integration, scholarship of application, scholarship of translation and scholarship of teaching. The latter translational research and the interface between laboratory research and public understanding of research are key areas that need to be addressed if we need to sustain the momentum in dental research.

Infrastructure Support

Faculty Shortages

There is a worldwide shortage of dental educators. For instance, in the 2004-2005 academic year, there were approximately 275 dental faculty vacancies in the United States — an average of 4.9 per dental school. In Hong Kong, too, we face similar challenges. A scholarly and adequately sized full-time dental faculty is essential to appropriately train future generations of students. This is also necessary in order to sustain the current level of research, so that the Faculty maintain its scientific standing and credibility in the university community both at local and international levels.

There are several reasons for the widespread shortage of dental educators. University salaries and benefits are not keeping pace with those in industry and private practice, and the gap is ever-widening. Hence, young graduates are attracted to the lucrative arena of general practice rather than face the challenges of academia. These challenges include competing for research grants, an ever-increasing administrative burden, and having to complete more and more training pathways prior to gaining specialist status. All of these activities restrict the time that can be devoted to scholarship. In the West, the shortfall in faculty

personnel is further exacerbated by graduates leaving dental school with a heavy burden of student debt that they want to unload as soon as is feasible by joining a financially rewarding private practice. Furthermore, attracting potentially high-earning dental specialists to a career in dental education continues to be an issue. Well-trained, scholarly dental specialists will be increasingly crucial to help dental schools maintain first-class teaching facilities.

Dental Facilities

Keeping pace with today's escalating technological advancements is a big challenge for most dental schools worldwide. The new technologies produce new machines such as lasers and modern imaging machines (e.g. cone-beam radiology) that provide precise images of hard and soft tissues. Yet, these are beyond access to resource starved dental schools. However, it behooves us to ensure that our dental students graduate with knowledge of and skills in using technology and equipment that are not already obsolete in the field.

Access to Care

Despite being an affluent society, Hong Kong unfortunately does not have free access to dental care for the whole community. The Dental Hospital and the Faculty of Dentistry play a major role in filling this gap in access to dental care, especially for those who cannot afford the sometimes high dental fees. Although there is some degree of Faculty involvement in community-based clinics and elderly homes, improving access of low-income groups to oral health care is indeed a challenge that Hong Kong has to face. For example, Hong Kong has ever-increasing numbers of edentulous (completely toothless) elderly in the community who are unable to afford expensive dental implantation treatment, as well as elderly with teeth who cannot afford routine care that is required to maintain healthy dentition.

CONCLUSION

The university of Hong Kong Faculty of Dentistry is celebrating its Silver Jubilee this year after 25 years of service to the community which has supplied one half of some 1900 dentists in Hong Kong. Hong Kong's dental education has reached maturity while maintaining its youthful vigour, and unfailingly provided dental education, research, and services to the community. However, the issue of dental education in Hong Kong is a complex one that has many shades of opinion regarding possible solutions to current and future problems.

It is clear that the challenges facing dental education in Hong Kong are very similar to those worldwide. These challenges will not be adequately addressed with anything less than a concerted response from the government, community, alumni, and industry. Only then can the Faculties or Schools of Dentistry continue to provide a stimulating and quality environment for graduates to develop their capabilities and to hone their competitive advantage for a challenging career in a fast-changing world.

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Dental Education in the United States: Responding to Challenges

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ABSTRACT

Dental education in the United States has reached a high level of excellence and standardization, owing in part to the accreditation process of the Commission on Dental Accreditation (CODA), other credentialing mechanisms, strong faculty, and the high caliber of student applicants. At the same time dental education faces substantial challenges related to disparities in oral health for underserved populations, the economic and policy environment for dental schools, and dental education's relative isolation from the rest of health professional education. There is also an explosion of new scientific information, and the need to graduate dentists who can be lifelong learners, including those who will contribute to the academic enterprise through research and teaching. The dense and prescribed curriculum in most dental schools, and the focus on technical competencies, makes it difficult to introduce educational reforms. Fortunately there are many innovations and opportunities that are helping to define pathways through these challenges. This paper reviews the basic requirements for dental graduates in the United States, societal trends and the response of dental education to these challenges. Examples of curricular innovations that we believe will help ensure that dentistry remains strong and vital in the changing health care arena are discussed.

Key words: dental education, curriculum, oral health

STRUCTURE AND ORGANIZATION OF DENTAL EDUCATION IN THE UNITED STATES

There are some 56 dental schools within the United States (2007). While each has its unique character there are specific accreditation standards that each school must be aligned with in order to be granted a dental degree. These standards are developed by the Commission on Dental Accreditation (CODA), which operates under the auspices of the American Dental Association.

Each school undergoes accreditation review every 7 years. The "Commission's accreditation review process is based on nationally-accepted standards established to guide program administrators, faculty and staff in developing and maintaining acceptable quality in educational programs. These standards address areas that directly affect the quality of dental and dental-related educational programs. These areas include outcome assessment, administration, educational programs and curriculum, faculty, financial support and physical facilities. The accreditation standards, which reflect input from a broad community of interest, are validated and revised periodically and constitute the current nationally accepted standards for dental and dental-related education. Accreditation actions taken by the Commission are based upon these standards." (American Dental Association: Commission on Dental Accreditation, 2007)

In addition there are National Board Dental Examinations (Parts 1 and 2) taken during dental school (American Dental Association, 2007a; American Dental Association, 2007b) and state/regional licensing exams that are required for individuals to receive a dental license.

Table 1: Dental Education in the United States

1A: Basic Requirements for Licensure

Licensure Requirements

- Pre-dental education (4 years)
- Dental School (4 years)
  - National Board Exams (Part I & II)
  - Clinical Board Exam (4th year of Dental School)

Dental Curriculum

- Years 1 & 2
  - Classroom and laboratory courses (including simulation models to build manual dexterity skills).
  - Curriculum topics: anatomy, behavioral sciences, biochemistry, histology, microbiology, pathology, physiology, pharmacology, ethics and professionalism. Some of these topics may be taught as integrated sciences and/or using a problem based approach.
  - Clinical outreach or research experiences (most schools)
- Years 3 & 4
  - Focus on patient care, hospital and community rotations
  - Elective courses – didactic and clinical (specialty areas- see table II)
  - Continued didactic courses – may include problem-solving/case-based learning

1B: What happens after dental school graduation? Various options

Private / Community Practice

Post graduate training

- General Practice Residency (GPR)
- Advanced Education in General Dentistry (AEGD)

(GPR focus is more oriented toward hospital – medically compromised patients vs. AEGD)

Dental specialty training

- Combined dental specialty/PhD programs –Academic programs
- Dental Public Health
- Endodontics
- Oral and Maxillofacial Pathology
- Oral and Maxillofacial Radiology
- Oral and Maxillofacial Surgery (to include DDS/MD)
- Oral Medicine
- Orthodontics and Dentofacial Orthopedics
- Pediatric Dentistry
- Periodontics
- Prosthodontics

Table 1 (1A) outlines the educational and licensure process in the United States and general topics required for graduation. Upon completion of this 4 year program (with one exception - University of Pacific is a 3 year program) the desired skills include patient-centered care, management of patients who are medically compromised or have disabilities; advanced diagnostic techniques and advanced restorative and prosthetic procedures. Table 2 summarizes the CODA standards, including curriculum.

Table 2: CODA standards for dental education

CODA Accreditation Standards	**CODA Education Program (Standard 2)
1. Institutional Effectiveness includes outcome assessment, administrative process	Curriculum Management Plan
2. Educational Program**	Biomedical Sciences
3. Faculty and Staff	Behavioral Sciences
4. Educational support services	Practice Management
5. Patient care services	Ethics and Professionalism
6. Research program	Information Management & Critical Thinking
	Clinical Sciences

* American Dental Association: Commission on Dental Accreditation, 2007

About 30% of graduates from dental schools in the United States receive further education in programs ranging from one year in length (Advanced Education in General Dentistry (AEGD) and General Practice Residency (GPR)) to five years or more for specialty training, or for other advanced degrees (e.g., MD, PhD, JD). Table 1 (1B) outlines the post graduate/specialty programs offered within the United States. Most of the remaining graduates go into private practice or work in community or public health clinics.

While this process provides important guidelines to assure quality of our dental programs across the country, as with any organization, as the environment changes there is a need to review the process and to readjust the process - some times significantly - to ensure that new developments are incorporated into the process. Dental educators in the U.S. recognize that substantial changes are needed in order for us to respond to three major issues: 1) the growing disparities in oral health and access to care among underserved groups in the U.S.; 2) the exponential growth in new scientific knowledge - including evidence for the importance of oral health to systemic health; and 3) the isolation of dental education from the other health professions at a time when interprofessional collaboration should be increasing.

Current and Emerging Societal Trends

These issues remind us that we live in a rapidly changing environment. We must continually assess and respond to new scientific information as well as societal trends if we are to be effective and recognized leaders in the health community. These trends - many of which are global trends - will shape our programs, curricula and policies in the years to come.

Disparities

In 2000 the U.S. Surgeon General - the chief medical officer of the United States - released a landmark report on oral health that underscored the importance of oral health to an individual's total well being, both emotionally and physically (U.S. Department of Health and Human Services, 2000). The report documented the substantial burden from oral diseases, a burden that is born unequally by minority and socioeconom-

ically disadvantaged groups. In spite of the strides that have been made in the United States to address access issues, there remains a large discrepancy between those individuals receiving adequate oral health care vs. those with limited or no access to oral health care. Dental Schools must take a leadership role in addressing the health care needs of communities via modifications in the dental curriculum to include emphasis on social responsibility and community service, and through education of other health professionals. Significantly, the Surgeon General also called for a revamping of all health profession's curricula to include oral health (U.S. Department of Health and Human Services, 2003).

Demographics

The evidence for disparities in oral health is coupled with the fact that as our population is growing, it is becoming increasingly diverse. Dental professionals of the future must be prepared to serve a culturally diverse patient population with a variety of values, life experiences and health practices. Individuals from minority groups are at increased risk for disparities for many reasons, including, among others, stereotyping and attitudes of health professionals (Smedley *et al.*, 2003). It is critical that our dental graduates exhibit a high level of cultural competency and an ability to communicate across different cultures.

The aging of our population presents another critical issue. Many elderly persons have significant medical conditions, and/or are receiving medications or other treatments which pose threats to oral health. Still others will have decreased capacity to care for themselves or will be in nursing homes or other facilities where their oral health needs may not be closely monitored. Dentists, physicians and other health professionals of the future will need an in-depth understanding of oral-systemic linkages and an ability to work collaboratively as members of the same health team. Geriatric training programs in dentistry are few and far between, yet the elderly are the most rapidly expanding segment of the population. Many of the same concerns apply to individuals with intellectual and physical disabilities, who also have oral health needs which may not be easy to assess or meet. These patients in particular may benefit from interdisciplinary team care, yet few dentists are prepared for this kind of practice.

Emerging Science and Technologies

New scientific knowledge and technologies are emerging daily. Areas of great progress include basic, clinical and translational research discoveries. Taking an example from my own field, the latest research linking bisphosphonates (used for treatment of individuals with osteoporosis, Paget's disease, metastatic prostate and breast cancer, and other diseases of bone homeostasis), to osteonecrosis of the jaw reveals just how critical is the need to continue research in oral-systemic areas (Wang *et al.*, 2007; Woo *et al.*, 2006; American Association of Oral and Maxillofacial Surgeons, 2007). Other examples of expansions in scientific knowledge and technologies include advances in biomaterials; in implants, computer and imaging technologies; and in approaches to diagnostics, risk assessment and prevention (Iacopino, 2007).

Yet much remains to be done, particularly in interdisciplinary research that may consider, for example, the biological, social, cultural and environmental factors which underlie differential disease rates. Despite enormous progress against caries in much of the population, existing data point to increasing caries rates among our nation's youngest children (15.2% increase in children age 2-5) (Beltran-Aguilar *et al.*, 2005). The National Institutes of Health (NIH) have given interdisciplinary and collaborative science a high profile with the funding of the new Clinical and Translational Science Awards (CTSA) (National Institutes of Health, 2007). The new practice based research networks of the National Institute of Dental and Craniofacial Research, NIH (Northwest Practice-Based Research Collaborative in Evidence-Based Dentistry ("Northwest PRECEDENT") for example) are helping ensure that research questions are relevant to community dentistry, and that the resulting data feed back the latest evidence to enhance clinical care (National Institute of Dental and Craniofacial Research, 2005).

To continue all this important work will require that we graduate more dental researchers to continue and expand the scope of dental research. It also requires that we graduate clinicians who will exercise life-long learning and use critical thinking skills, continually assessing and translating new discoveries into evidence-based practices. The pace of scientific change demands this, and we are assessing our educational programs with these goals in mind. Practitioners are the intermediate consumers of science, and we must ensure that they are prepared for this task.

Accountability and Quality Improvement

Across the health sector - as across our society in general - there is an increasing consumer demand for accountability and transparency, spurred at least in part by reports of medical error (Institute of Medicine, 2001; Gallagher *et al.*, 2007). Part of accountability practice includes continually assessing and improving our patient care, educational systems, and community programs. These needs are impacting how we do business in our clinics, in our classrooms and in the community.

Professionalism/Ethics

Examining ones biases in order to respond appropriately to patients of different cultures, holding our profession accountable for high standards of patient-centered care and taking action on social injustices in the health care delivery system are all examples of professionalism. So also is the accountability for our health research enterprise that includes maintaining ethical standards of scientific conduct as well as ensuring our scientific discoveries are translated into practices that improve the health of patients and the public at large. Across medicine and dentistry and

the other health professions there is increased attention to the attitudes, knowledge and skills associated with professionalism, with recent efforts being advanced by the ADA (American Dental Association, 2007), American Dental Education Association (ADEA) and others. Yet it may be difficult at times to translate clinical ethical dilemmas across cultures and societies where health beliefs and health practices vary widely, and where health systems are organized so differently. There is a need for more discussion of ethical issues in global forums such as this, the 2nd Hiroshima Conference on Education and Science in Dentistry.

Workforce: Numbers, Distribution and Capacity

And what can be said about the ability of our dental workforce to meet these challenges? Many dentists are reaching retirement age in the US, but perhaps more important are the questions of distribution and capacity. In rural and remote areas - of which there are many in the Pacific Northwest- there are a large number of dental professional shortage areas. This applies to inner city urban areas as well. Capacity includes dentists' ability to meet the current and emerging needs of our society, which we must address through curriculum reform and continuing education. Another key aspect of workforce capacity is diversity: we must recruit and train dentists who are more representative of the changing society. Many efforts are being dedicated to increasing the "pipeline" of dental professionals from underserved groups.

OBSTACLES TO MOVING FORWARD

If our profession is to continue be a leader in health care, we must transform our system of dental education to respond to the three major issues identified above: disparities, new scientific knowledge, and the isolation of dental education. Change is difficult in any system, but there are particular factors that work against change in dental education.

To respond to **oral health disparities**, for example, we will need dentists who are socially responsible, with a high sense of professionalism, proactively involved in improving the oral and overall health of all groups of patients. In addition to possessing a strong moral compass, which we are most proud of as a profession, dentists must be well-trained in the behavioral sciences, with good communication skills, and culturally-sensitive to the changing patient population around them. Yet it is difficult to include all the educational reforms needed for this emphasis, including clinical experiences in underserved communities, within the existing densely-packed curriculum that stresses memorization and technical skills.

This approach to education also impedes us from moving forward with reforms needed to address the second major issue, the **exponential growth in new scientific knowledge**. Students need to develop critical thinking skills and the ability to analyze and apply new scientific information. The strong procedural focus makes this difficult to accomplish. This focus may be necessary to some extent, to achieve the high level of technical proficiency we expect from students in four years, but we must find ways of integrating the newer skills into the more traditional educational competencies of dental students. Increased attention to new scientific information and evidence-based practices in the dental curriculum may also help us in recruiting students to our research programs.

This brings us to another difficulty we are facing - **the shortage of faculty** and the low numbers of dental students choosing academic careers. Students' expectations of the life style and rewards (or lack thereof) of the academic world may contribute to the shortage of faculty. In fact, dentists are doing very well in the U.S., and can continue to do so by providing a lucrative kind of dentistry in private practice. This tendency reflects the larger trend of commercialism in the health "industry" in the U.S., and the demand in particular for cosmetic dentistry and other costly procedures. It should be acknowledged that many dentists who are provide these "high-end" serv ices also volunteer their time caring for the underserved. The problem is that these volunteer efforts may help individual patients, but do not constitute a system of care; they are more a "band-aid approach" (Mouradian, 2006). To the extent that dental schools themselves must limit their care for underserved patients due to fiscal shortages, dental students receives mixed messages about care for poorer patients.

Fiscal shortages are due in part to lack of public funding for dental education and dental care, and reflect another major obstacle - **health policies that do not value oral health**. While there has been movement in this direction since the Surgeon General's Report, it has not been sufficient. We are a long way from boasting an equitable system of healthcare in the U.S., but the discrepancy is even greater for the underserved when it comes to access to dental care. Since dentistry and medicine have for the most part had separate systems of healthcare, funding and education, it is no surprise that many policymakers are unaware of the importance of oral health. Frequently the oral health community is not at the policy table when key allocation decisions are made. Yet even this is changing.

Perhaps one of the most important challenges for dental education is to address its relative **isolation from the rest of the health care system**. In addition to increasing the awareness of the importance of oral health among other health professionals, policy makers and the public, greater integration with the other health professions will help address the increasingly complex health needs of patients with chronic illnesses and medical conditions. The new data on oral-systemic interactions make it clear that when we ignore these linkages we put patients at risk of poorer health outcomes. Dentists of the future must be able to participate as members of the health care team, and this will not happen unless we dramatically alter our isolationist approach to educating dentists.

To enact these many changes we must utilize effective change management strategies that engage our

faculty, build upon strengths of the current dental education system, enhance curricula in new and creative ways, while preserving those portions that work well. This will not be easy, but there is a will to do this, and much is happening at the national level to support dental schools in these efforts.

DENTAL EDUCATION'S RESPONSE

Emerging Standards and Competencies

Professionalism in dental education includes updating our educational practices and standards to match society's complex needs. A critical step for ensuring that our graduates are equipped with the knowledge, skills and attitudes required to serve the oral health needs of the public is to define the dental educational environment essential for achieving the desired outcome at graduation from dental school. The American Dental Education Association, Commission on Change and Innovation in Dental Education (ADEA/CCI) has taken a leadership role in this effort by defining eight basic principles essential for creating such a dental educational environment. These principles, outlined below (see also Table 3) directly respond to the emerging societal trends considered above. For an in-depth discussion on these principles please see Haden *et al.*, 2006.

1. Critical Thinking
2. Lifelong and Self directed learning
3. Humanistic Environment
4. Scientific Discovery and the Integration of Knowledge
5. Evidence Based Oral Health Care
6. Assessment
7. Faculty Development
8. The Health Care Team

These eight basic principles are a good beginning - they establish a framework for the needed changes that must happen in order for us to make sure that our graduates play a vital role in improving not only the oral health but the total health of the nation. Although each school will develop programs unique to its school environment, there are some common themes set forth under these eight principles that are applicable to all schools world-wide, and that we need to work on together in order to educate life long learners to provide patient centered, evidence based care for all members of our society.

Linked with the basic framework for change are the competencies that our students must have at graduation to meet the challenges facing the new graduate. In their current format these include:critical thinking, professionalism, communication and interpersonal skills, health promotion, practice management and informatics, and patient care (Table 3 and American Dental Education Association, Commission on Change and Innovation in Dental Education, 2007). Although some of these competencies are currently included within CODA standards, the CCI is examining whether further articulation and discussion of these standards can assist dental schools in enhancing these educational attainments. Significantly these competencies are in keeping with other current formulations of health professional competencies such as the IOM's *Health Professions Education: A Bridge to Quality* (Greiner *et al.*, 2003): Provide patient-centered care; Work in interdisciplinary teams; Employ evidence-based practice; Apply quality improvement approaches, and Utilize informatics. These domains also overlap with the Accreditation Council on Graduate Medical Education (ACGME) educational competencies for the training of physicians: Patient care, Medical knowledge, Practice-based learning and improvement, Interpersonal and communication skills, Professionalism and systems-based practice. There is a need for interprofessional and international discussions to flesh out the competencies that will guide our educational systems into the future. One way we could do this would be to develop a web-site for sharing international competencies and the educational innovations and assessment approaches underway in different settings, similar to the ACGME Outcomes Project for medical schools in the U.S. (Accreditation Council for Graduate Medical Education, 2007).

Table 3: Dental Educational for the Future (ADEA - CCI)

ADEA Principles for Dental Education Environment*	ADEA Commission on Change and Innovation: Competencies**
Critical Thinking	Critical thinking
Lifelong and Self directed learning	Professionalism
Humanistic Environment	Communication and interpersonal skills
Scientific Discovery and the Integration of Knowledge	Health Promotion
Assessment	Practice management and informatics
Faculty Development	Patient Care
The Health Care Team	

* Haden NK, Andrieu SC, Chadwick DG, Chmar JE, Cole JR, George MC *et al.* (2006) The Dental Education Environment. *J Dent Educ.* 70:1265-1270

** American Dental Education Association, Commission on Change and Innovation in Dental Education (2007). http://www.adea.org/cci/CallforComments09292006.pdf

Educational Strategies

With the new rapid pace environment we are facing we must provide learning environments that do more than transfer knowledge -we must "educate" as well as teach. Education is the process of learning through interactions, engagements, discourse, critical analysis - that will develop curious minds, creative thinkers and openness to new approaches to clinical performance. If we do this successfully the result will be the development of the "master clinician," those individuals that are always seeking, always anxious as appropriate to embrace new knowledge and new technologies. Some examples of educational strategies that address competencies for the future health professionals are listed in Table 4.

Table 4: Educational Strategies to Achieve New Competencies

Educational Issue	Some Possible Strategies
Critical thinking, evidence-based care	Problem-based methods, evidence-based review of scientific literature, classroom and small group discussions, research opportunities
Professionalism / ethics (including social responsibility)	Increased emphasis on professionalism in all curricular elements, especially clinical experiences; "White-coat" and other ceremonies and "profession" of ethical codes analogous to Hippocratic code; "ethics rounds; case-based and small group discussions; faculty and practitioner case examples; interdisciplinary groups with other health professional students; journaling and self-reflective activities; rotations in underserved and culturally diverse communities
Communication and interpersonal skills (including cultural competency)	Experiential learning, role-playing/simulations, journaling, peer and faculty observations, objective standardized clinical examinations (OSCE's); emphasis on behavioral components in oral and overall health outcomes; rotations in underserved and culturally diverse communities
Practice management	Course offerings, problem-based and case methods, real-life experiences provided at the School, and with community practitioners and public health clinics (e.g. senior year)
The health team – interprofessional collaboration	Interdisciplinary discussions with other health professional students, guest faculty from other professions in courses and "Grand Rounds", opportunities to participate as member of health team on hospital ward or outpatient clinic, mentoring other health professional students (eg, medical students learning oral health exam and patient counseling), interdisciplinary research opportunities

Beyond designing learning environments more conducive to inquisition, exploration, creative thinking we must provide environments that are a model for the highest level of ethical and professional behavior for our faculty, staff and students. Schools must take a leadership role in this effort through a commitment to: patient-centered clinical experiences; addressing issues around the disparity in access to care for specific populations; presenting and openly discussing ethical dilemmas with our students and expecting the highest ethical and professional behavior for the school as a community. An excellent discussion of professionalism in dentistry was presented by Masella *et al.* where they refer to professionalism as "a caring and humanitarian activity that respects patients and colleagues and strives to give something back to community and profession" (Masella *et al.*, 2005; Masella *et al.*, 2007).

Important aspects for promoting professionalism include a culture at the School that sets professionalism as a daily expectation; that has strong professional role models; that has a strong commitment to community service; and that provides a humanistic environment, one that "inculcates respect, tolerance, understanding and concern for others" (Haden *et al.*, 2006). See Table 4 for educational strategies for promoting professionalism/ethics.

Interprofessional Collaboration

The last principle listed above for the ideal dental educational environment, the Health Care Team, is one in which the dental community needs to play a more active role. Simply put, it takes a "village" - an entire health team - to address the needs of patients - especially those from underserved communities or who have complex health conditions; just as it takes a team for complex research projects, or for holistic and engaging educational practices. All the components - dental, medical, social, environmental issues contribute to health outcomes. Our professional education systems have been slow to respond to this mandate, slow to model the attitudes and behaviors of interprofessional collaboration, and slow to utilize the potential of dentists in overall health maintenance. As members of the team, our graduates can be the primary health care resource for oral health care, as well as for the screening of a wide variety of disease that may be detectable within the oral cavity or with use of oral tissue/fluid (saliva) markers for systemic diseases. Dental schools will need to provide much stronger programs in a variety of areas - including basic sciences, behavioral sciences, and in diagnostic technologies, if we are to accomplish this objective.

University of Washington, School of Dentistry (UWSoD) Educational Innovations

The UW, along with many other dental schools, pairs medical and dental schools together for basic sciences education. However we are expanding efforts beyond this to deliberately increase the interaction of dental students with other health professional students, while simultaneously increasing the exposure of these other students to oral health issues (Mouradian *et al.*, 2005, 2006). Other innovations include community-based rotations in rural and underserved areas with expansion to develop regional models for dental education (http://dental.washington.edu/ride); curricula modifications to include interdisciplinary and focused opportunities for students and faculty to review the scientific literature or engage in research across a variety of basic, clinical and public health topics; practice based networks for research (PRECEDENT-https://clinicaltrialsworkbench.axioresearch.com/nwprecedent/) and international grants providing for faculty development in research internationally.

PARTNERSHIPS

We can learn much from each other. In the U.S. the persistence of health disparities in the face of enormous progress in science and new technologies will find parallels in other countries. The new environment we are presented with mandates that we collectively define new, innovative models for educating the next generation of dental professionals. It is hoped that this review of critical issues in dental education in the U.S. will spark discussion and debate and lead to better understanding, and to a commitment to work together on developing programs to enhance the educational needs of our students and the oral health needs of society.

ACKNOWLEDGEMENTS

We would like to thank the organizers of 2nd Hiroshima Conference on Education and Science in Dentistry for inviting us to participate in this innovate conference that fosters a dialogue between education

and research in dentistry. We would also like to thank our colleagues at the University of Washington for their continued support of our educational programs. Special thanks to Jennifer Grant and Amelia Brett Connelly for their assistance in the preparation of this manuscript.

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Dental Education in Malaysia

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ABSTRACT

This paper provides information on the training of dentists in Malaysia. It defines the practice of dentistry under the Dental Act and presents the current status on the number of practitioners in Malaysia. The various institutions offering dental degree programmes are listed. It focuses on the dental degree programme at the Faculty of Dentistry, University of Malaya which is the oldest dental institution providing undergraduate dental programme in Malaysia. The admission requirement as well as the course structure and teaching methodology undertaken are presented. The weaknesses of the present curriculum are identified and a new curriculum is being planned. The philosophy and aim of the new integrated curriculum is spelt out and the structure of the integration in the new curriculum is provided. The postgraduate courses offered by the Faculty of Dentistry, University of Malaya are listed. The proposed mandatory continuing dental education programme for dentists which is currently under trial is also presented.

Keywords: Dental education, dental curriculum, dental practice, Malaysia

Background of Malaysia

Malaysia is situated in South East Asia. It consists of Peninsular Malaysia and the states of Sabah and Sarawak (costal area of Borneo Island). The peninsula has its frontiers with Thailand in the North and Singapore in the South whereas Sabah and Sarawak are located above the border territory of Indonesia Kalimantan province. These two regions are separated by the South China Sea.

Malaysia is blessed with diverse multiracial cultures and rich heritages. It comprises of different racial communities with the Malays, Chinese and Indians forming the main races and the indigenous people of Sabah and Sarawak. Based on Census 2000 (Department of Statistics, Malaysia, 2000), Malaysia has a population of 23.3 million people and still growing at an average rate of 2.3% per annum. The distribution of total population in Malaysia comprised of 65.1% 'Bumiputera' (Malays and native indigenous groups), 26.0% Chinese and 7.7% Indians and other ethnic minorities.

The Practice of Dentistry in Malaysia

A person shall be deemed to practice dentistry within the meaning of the Dental Act of Malaysia (Government of Malaysia, 1971), for the sake of gain or otherwise if he treats or attempts to treat or profess to treat, cure, relieve or prevent any oral disease by undertaking any measures towards achieving this end. A person is also deemed to practice dentistry if he holds himself out directly or indirectly as practicing dentistry.

Any person who wishes to practise dentistry in Malaysia must have his name registered in the Malaysian Dental Register maintained by the Registrar of Dental Practitioners. The Register is kept in two Divisions as follows:

i) Division 1 which shall be in respect of persons registered as dental surgeons. (To conform to international terminology, they will subsequently be referred to as dentists). This applies to those holding any of the qualifications sp ecified in the Dental Act granted by recognized institutions.

ii) Division II which shall be in respect of persons registered as dentists. (They will subsequently be referred to as Division II dentists). This applies to those who immediately before the coming into force of the Dental Act was registered as a dentist in the division two of the register maintained under the Registration of Dentists Ordinance 1948 of West Malaysia; or as a dentist by virtue of the Dentists Registration Ordinance of Sarawak; or was actually engaged, as his sole means of livelihood, in the practice of dentistry in the State of Sabah for a continuous period of three years immediately prior to the coming into force of the Dental Act. In contrast to the Dental Surgeons, the Division II dentists do not have a formal dental education.

The Dental Act was amended in year 2003 for national purpose with the provision that every person who obtains registration shall be required to serve as a dental officer in the public service to the satisfaction of the Director General of Health for a continuous period of not less than three years. However the Minister of Health has the power to grant reduction, exemption or postponement from this period of service.

Training of dentists in Malaysia

Up to the early 1970s, most of the dentists in the country were graduates from Singapore but their numbers are limited by a quota system. With the establishment of the Faculty of Dentistry in Malaysia in 1972, the number of Malaysian graduates from Singa-

pore gradually declined, to the extent that today practically none comes from that country.

At the end of 2005, there were 3837 practitioners registered with the Malaysian Dental Council. Of these, 2743 were active and were issued with Annual Practicing Certificates (APCs); 2675 to Division I practitioners and 68 to Division II practitioners. Overall there are more dental practitioners in the private sector (56.6%) and the majority (55.4%) comprised of female practitioners. In terms of service sectors, almost three-quarters of dental practitioners in the public sector are females as compared to about 40% in the private sector. The number of Division II dentists in the register is steadily decreasing and they only work in the private sector. In 2004 there were 68 APCs issued to Division II of which only six were females (Malaysian Dental Council, 2006).

Institutions offering Dental Degree programmes

At present there are 9 institutions of higher education offering dental degree courses. Of these, six are public universities i.e. Universiti Malaya (UM), Universiti Kebangsaan Malaysia (UKM), Universiti Sains Malaysia (USM), Universiti Teknologi Mara (UiTM), International Islamic University (IIU) and Universiti Sains Islam Malaysia (USIM). There are 3 private institutions - the Asian Institute of Medicine, Science and Technology (AIMST), the Penang International Dental College (PIDC) and the Malaysian Allied Health Science Academy (MAHSA). The location of these institutions, their year of establishment, graduating year and present intake is shown in Table 1. The degrees from the last six dental institutions have not been recognized yet, as the Malaysian Dental Council will only consider a degree for recognition in the fifth year. However the Council monitors closely the progress in the development of these dental institutions in their earlier years.

Institution	Location	Year of First Intake	Year of First Batch of Graduates	Present Intake
UM	Kuala Lumpur	1972	1978	80
UKM	Kuala Lumpur	1997	2002	65
USM	Kelantan	1999	2004	60
AIMST	Kedah	2005	2010	50
UiTM	Selangor	2006	2011	32
PIDC	Penang	2006	2011	50
IIUM	Pahang	2007	2012	50
USIM	Kuala Lumpur	2007	2012	30
MAHSA	Kuala Lumpur	2007	2012	50

Table 1.
Institutions providing dental degree programme in Malaysia

Dental Education at the Faculty of Dentistry, University of Malaya

The Faculty of Dentistry, University of Malaya was established in 1971. Initially led by an Administrative Dean, 32 pioneer students enrolled for the 1972/73 academic session for the 4-year course in dentistry leading to the degree of Bachelor of Dental Surgery (BDS). Being the first local dental school, the University of Malaya Dental Faculty undertook the role of training undergraduate students, a function that was then fulfilled by overseas universities. This was subsequently extended to specialty training in various disciplines.

The Faculty of Dentistry's mission is to be a centre of excellence in oral health sciences, education and research and to promote learning and research in dentistry. Its vision is to be a premier faculty providing a high standard of training and research to produce caring dental professionals with holistic approach

The faculty received recognition for its high standard of teaching, research and professionalism when the General Dental Council of the United Kingdom gave its recognition to the University's Bachelor of Dental Surgery degree in 1997. The Dental Faculty is also recognized by the Royal College of England as a training hospital fulfilling the clinical requirements needed to sit for the Membership of the Faculty of Dentistry (MFDS) Examinations. The Royal College of Surgeons of England also conducts the MFDS examinations at the Faculty, one of the few places where the examinations are conducted outside the United Kingdom.

Admission Requirements for the BDS programme

To be considered for admission to the Faculty of Dentistry, a candidate must fulfill the general academic requirements of the University as well as the special academic requirements of the Faculty (Faculty of Dentistry, 2007).

A candidate must possess the Sijil Pelajaran Malaysia (SPM) or the Malaysian Certificate of Education (MCE) or any other equivalent qualifications approved by the University Senate. The candidate must have passed in at least four subjects with credit. In addition the candidate must have obtained the Sijil Tinggi Persekolahan Malaysia (STPM) or the Higher School Certificate (HSC) not earlier than two years after obtaining the earlier qualifications. As an alternative to the STPM/HSC, candidates who have joined the matriculation course in the University of Malaya or that offered by the Ministry of Higher Education can be considered for admission in Year I of the course. Such candidates must also fulfill the special requirement of the Faculty by having a pass with a minimum of B grade in Biology and pass with a minimum of B grade in 2 of the subjects as listed below:

- Physics
- Chemistry
- Mathematics

Student intake

When the Bachelor of Dental Surgery was first offered by the Faculty of Dentistry, University of Malaya in 1972, the initial intake was 32 students. The intake was progressively increased to 48 in 1979, to 64 in 1982 and subsequently to 80 in 1991. Prior to the academic year 2003/2004, the selection process was by based on quota system as determined by the ethnic composition of the country. From the academic year 2004/2005 onwards, the selection of students was entirely based on merit. About 75% of the current batch comprised of females.

Programme duration

The duration of study for the Bachelor of Dental Surgery degree in University of Malaya was 4 years during the year 1972 to April 1995. Beginning in July 1995, the programme was extended to 5 years; comprising of two preclinical and 3 clinical years. Students are required to complete the preclinical years within three years and the Clinical years in five years. Hence the maximum duration to complete the course is eight years.

Course Structure

During the preclinical years i.e. Year 1 and Year 2, students are taught basic medical and preclinical sciences in collaboration with the Medical Faculty of University of Malaya. In Year 2, clinical skills in Dentistry are first taught through laboratory work in which students practice their skills on phantom heads.

During the clinical years i.e. Year 3, 4 and 5, the students are exposed to treating patients under closed supervision by the lecturers. The students practice 4-handed dentistry, with a clinical partner who is a course mate in the same academic year. In Year 3, students are required to pass Human Disease as one of the core subjects. This is to emphasize the relationship between oral health and the total health and wellbeing, besides understanding the role of doctors and dentists in working as a team. In Year 4 and 5, the course totally focuses on the study of Dentistry. The subjects taught and examined in the existing curriculum in the different years of study are shown in Table 2.

Besides developing clinical skills amongst the students, the curriculum also focuses on the development of inter-personal skills, the ability to evaluate and criticize clinical scenarios and the importance of research findings. Students are trained to be competent individuals in coming up with precise diagnosis and management, providing the best appropriate treatment and care, as well as in prevention of oral diseases and providing health education and information to the public. Various programmes and activities such as the Final Year Elective Project, Behavioural Science Project. School Dental Health Day Project, Problem Based Learning, seminars and tutorials are implemented to produce quality graduates.

Year 1	Year 2
Anatomy # Physiology# Biochemistry# Oral Biology#	Medical Microbiology# Pathology# Pharmacology# Dental Materials and Technology# Community Dentistry
Year 3	**Year 4**
Human Disease# Oral Pathology# Conservative Dentistry Prosthetic Dentistry Community Dentistry Oral Maxillofacial Surgery & Radiology Oral Medicine & Periodontology Children's' Dentistry and Orthodontics	Community Dentistry# Oral Maxillofacial Surgery & Radiology# Oral Medicine & Periodontology# Conservative Dentistry Prosthetic Dentistry Children's' Dentistry & Orthodontics Elective Project
Year 5	
Conservative Dentistry# Prosthetic Dentistry # Children's' Dentistry and Orthodontics# Elective Project General Dental Practice Oral Maxillofacial Surgery & Radiology Oral Medicine & Periodontology Community Dentistry	

Subjects examined at the end of the respective academic years

Table 2.
Subjects taught and examined in the existing curriculum

Teaching

Teaching is undertaken through lectures, tutorials, seminars, practical sessions and laboratory work. Students will start learning and practicing skills on Conservative Dentistry and Prosthetic Dentistry through laboratory work done on phantom heads.

Problem-based learning (PBL) is an educational approach that organizes curriculum and instruction around carefully crafted ill-structured problems. Students gather and apply knowledge from multiple disciplines in their quest for solutions. Guided by lecturers acting as cognitive coaches, they develop critical thinking, problem solving, and collaborative skills as they identify problems, formulate hypotheses, conduct data searches, formulate solutions and determine the best "fit" of solutions to the conditions of the problem. Problem-based learning enables students to embrace complexity, find relevance and joy in their learning, and enhance their capacity for creative and responsible real-world problem-solving.

PBL session in the faculty is conducted in a group of 10 to 14 students, guided by a lecturer or facilitator. For each of the PBL module, the group will meet two or three times, depending on the topic. During the first session, the group will be given 2 hours to brainstorm and identify the problems. Case module is constructed as triggers that have to be discussed in order to identify the learning objectives. Students will then work independently to undertake research and gather information that will be discussed during the second and third session which is held a few weeks after. Students will be evaluated based on the amount of participation in the group. The group will then emerge into 2 groups to come up with a written report which will contribute towards the Continuous Assessment of the subjects.

Weaknesses identified in the current curriculum

1. The concept of total patient care should be one of the guiding principles of all clinical courses. Yet the present curriculum at University Malaya heavily subscribes to the traditional model where areas of practice are compartmentalized and the tooth has been taken as the unit upon which the profession should focus - a model which poses a number of problems. The compartmentalized organization of the teaching programme often resulted in student's failure to integrate different items of knowledge from other areas of study that may have an important bearing upon a particular clinical problem. The compartmentalized organization of the teaching programme and the student's performance criteria for each clinical area also tends to negate the 'whole patient' care. Although a General Dental Practice module has been introduced, it is insufficient to bring about full integration in the clinical years.
2. In meeting educational objectives, the curriculum adopts various teaching-learning approaches which includes didactic teaching, seminars, practical/-clinical, problem-solving and case studies, PBL, research project and report writing, community oral health posting and community oral health project. However, despite the adoption of various teaching methods, students still lacks the ability to critically appraise a scientific paper which is important to face future challenges of evidence-based practice of their future career.
3. The teaching of basic sciences is done mainly in Year 1 and 2, while the clinical education begins in Year 3 and spreads through to Year 5. With this approach, there is inadequate integration between the basic sciences and clinical education. Integration between various disciplines is being attempted via PBL modules but there is still a lack of integration between disciplines in Dentistry.

With these weaknesses identified, the current curriculum is being revised in order to address the weaknesses while maintaining its strength as well as to maintain its international benchmarking. A new curriculum is being planned.

Philosophy of the new curriculum

1. The Professional philosophy

The curriculum is committed to the training of general dental practitioners who are technically competent and socially sensitive; and in conjunction are able to utilize the basic principles of human biology and human behaviour in promoting, preventing, diagnosing and treating oral disease in individuals and the community. The graduate adheres to the highest standards of professional conduct and ethics and who can function effectively as a member of the nation's health care delivery system. A graduate of this curriculum is caring, knowledgeable, competent and skilful in providing holistic care; and accepts professional responsibilities for the effective and safe care of patients.

2. The Educational Philosophy

The educational philosophy emphasizes a competency-based curriculum with emphasis on clinical relevance in the early years. Learning is facilitated within an integrated framework and important concepts are revisited in a "spiral effect" through all phases of the course. Problem-based, system-based, case-based and evidence-based approaches are introduced to encourage reflective learning, self-directed learning and sharpen problem-solving skills. It fosters critical thinking and lifelong learning for continuing professional development. Formative and summative integrated assessments in all phases reflect the philosophy of the curriculum. Overall, there will be alignment in the learning outcomes, teaching and learning activities and assessment methods to meet the aims of the curriculum.

3. Aims of the revised curriculum

The desired graduates attributes should be a caring, knowledgeable, competent and ethical dentist who:

1. is able to independently practice safely, effectively and efficiently;
2. is able to work together with other dental and healthcare professionals;
3. is able to utilize appropriately advances in relevant knowledge and techniques in patient care;
4. understands the role of patients and the community in making decisions about their health; and
5. engages in life-long learning and continuing professional development

Domains, learning outcomes and competencies

In order to meet these aims, the graduate must attain specific learning outcomes (LOs). The learning outcomes define the competencies (knowledge, skills and values) required to begin the independent practice of general dentistry. These LO's are grouped into 15 domains which are broad categories of professional activity. Closely related domains are further grouped into Themes to ease implementation. The various themes and the grouping of the domains in the new curriculum are shown in Table 3. The structure of the integration in the new curriculum is shown in Figure 1.

Themes	Domains	Topics
1	A. Man In Health And Disease	01. Introductory topics
		02. Birth, growth, and aging
		03. From molecules to life
		04. Signalling and communication
		05. Scientific basis of diseases and therapeutics – normal reactions in diseased states
		06. Scientific basis of diseases and therapeutics – body's reaction to chemical and biological agents
		07. Gastrointestinal health and diseases
		08.CVS and respiratory health and diseases
		09. Endocrine and reproductive health and diseases
		10. Blood and renal health and diseases
		11. Neurological and musculoskeletal health and diseases
		12. Infection
		13. Nutrition and diet in health and diseases
		14. Behavioural and Social factors contributing to health and disease
	B. The Mouth In Health And Disease	01. Normal structure and development of oral and peri-oral region
		02. Functions of the oral and peri-oral region
		03. Alterations to normal structure and function in disease
		04. Biochemical and microbiological basis of the oral environment
		05. The role of micro-organisms in the mouth in health and disease
		06. Impact of pharmaceutical agents on the functioning of the mouth
		07. Diet in dental health and disease
		08. Behavioural and Social factors contributing to oral health and oral disease
2	C. Scientific understanding and thought	01. Scientific principles
		02. Research methodology
		03. Evidence-based dentistry
	D. Communication skills	01. Communication skills
	E. ICT	01. Information and Communication Technology
3	F. Team-Working And Leadership	01. Team working
		02. Leadership
	G. Health Promotion And Disease Prevention	01. Health Promotion And Disease Prevention
	H. Management And Administration of Public Health Programs	01. Planning and Evaluation of Public Health Programs
		02. Management and Administration of Public Health Programs
4	J. Clinical Competence – Diagnostic Skills (including Imaging)	01. Diagnostic Skills
		02. Dental Imaging
	K. Clinical Competence - Manual skills and dexterity	01. Managing Dental Unit
		02. Injection Techniques
		03. Restoration of Teeth
		04. Periodontal Therapy
		05. Extraction and surgical removal of teeth and roots
		06. Surgical procedures in dentistry
		07. Occlusal Management
		08 Endodontic Therapy
		09. Imaging Techniques
	L. Clinical Competence - Problem Solving	01. Clinical competence problem solving
	M. Patient Management	01. Patient Management
	N. Practice Management	01. Planning and setting a dental practice
		02. Managing a dental practice
5	P. Professionalism	01. Professional Behaviour
		02. Ethics and Jurisprudence
	Q. Reflective Practices	01. Reflective Practices

Table 3. The proposed new curriculum

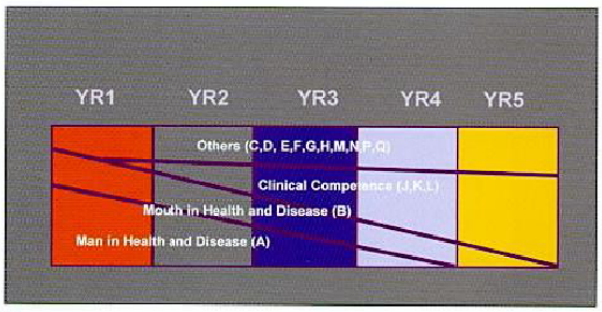

Figure 1.
Structure of the integration in the new curriculum

The challenges implementing the new curriculum

With this curriculum, the Faculty intends to train the 21st Century Dentists for Malaysia who are adaptable to change (who are critical thinkers, independent learners and evidence-based practitioners). However, there are challenges ahead in implementing the curriculum. Foremost would be bridging the concepts of the current and new curriculum that requires a tremendous change in mindset among the staff. The integrated curriculum must withstand pressure from the territorial nature of the existing departmental structure. How can faculty best reconcile these two opposing concepts? Moving into the new curriculum will also mean intensive use of human capital (intensive coordination among theme and year leaders to ensure vertical and horizontal integration) and monetary resources (such as for training staff). As in any change, it is expected that there will be resistance among some staff. There is a need to be mindful of these challenges ahead of us, to prepare us, soften the negative impacts and at the same time take advantage of opportunities that come along.

Postgraduate courses offered

The Faculty of Dentistry, University of Malaya also offers post graduate training in both academic and specialty training programmes (Faculty of Dentistry, 2006). These include

- Master of Public Health (Oral Health) - 4 years
- Master of Science (Dental Public Health) - 2 years
- Master of Clinical Dentistry - 4 years
- Master of Orthodontics - 4 years
- Master of Dental science - 1 year
- Doctor of Philosophy - 3 years

Mandatory Continuing Professional Education

According to the Dental Act of Malaysia, a dentist on qualification is presumed by law to be competent to carry out dental practice but is under no obligation to seek any form of updating of knowledge, skills or techniques. He or she could even reach retirement after a lifetime of practice without ever having attended a refresher course or kept up with the literature.

However, through their professional life, the dentists will need the judgement and capacity to adapt to changes in the patterns of health and disease, medications, new technologies, improved equipment and materials, more effective team co-operation and more demanding public expectation. This can only be attained through on-going learning throughout their professional lifes and not only until they are qualified to practice their chosen profession. Such opportunities must be made available to the dentists to maintain and improve their knowledge and skills and to learn of new advances

The Malaysian Dental Council which is the governing body of the dental profession in Malaysia has shifted its view towards lifelong education of dental practitioners. A periodic relisencing system is being considered. Course provider makes application and credit point is assigned. Only practitioners who have attained a minimum credit point will be granted with an Annual Practicing certificate by the Malaysian Dental Council. The system is currently under trial basis.

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Education of the School of Oral Health Science, Hiroshima University Faculty of Dentistry

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1. Hiroshima University Faculty of Dentistry

Hiroshima University Faculty of Dentistry consists of two schools, which provide 4 original and distinct courses for students.

One is the School of Dentistry to train dentist for six years, comprised of Scientific Research Course and Clinical Training Course, and the other one is the School of Oral Health Science to provide Oral Health Science Course and Oral Health Engineering Course, which cultivate the student to be dental hygienists and dental technicians for four years, respectively. And our faculty is the only one to have schools of all the professions that work in dentistry in Japan.

2. The System and Constitution of School of Oral Health Science

The School of Oral Health Science, Hiroshima University Faculty of Dentistry was established in 2005, developed from the Dental Hygienist School (since 1976) and Dental Technician School (since 1972) in the faculty. The school has two divisions, the Division of Oral Health Science and the Division of Oral Health Engineering, and the regular number of the students in each division is 20.

The School consists of 13 full-time staffs and each division has three departments as follows.

Division of Oral Health Science
- Department of Oral Health Research
- Department of Team Care for Oral Health
- Department of Oral Health Management

Division of Oral Health Engineering
- Department of Oral Basic Science
- Department of Biomaterial Engineering
- Department of Medical Design and Engineering

3. School of Oral Health Science

The outlook of curriculum at both Oral Health Science Course and Oral Health Engineering Course are shown in Figs. 1 & 2. In the 1st and 2nd semesters, the students learn the general education, i.e. wide-ranging studies (literature, foreign languages, communication, basic science, etc.) during the period in the main campus of Hiroshima University in Higashihiroshima City (for 1 year). Subsequently, they have the specific programs in Kasumi Campus for three years as below. From the semester 6 to 8, they have the clinical practice and training in the Hiroshima University Hospital. Concurrently, they start the studies for graduation thesis at this period.

Basic Subjects

Basic Oral Science : Anatomy, Physiology, Pathology, Pharmacology, Microbiology Immunology, Basic Practice of Oral Science etc.

Social Dentistry : Oral Health Science, Hygiene Administration, Social Welfare etc.

Related Medical Science : General Medicine, Medical Ethics, Exercise of Nutritional Instruction etc.

Specialized Subjects

Team Care for Oral Health : Dentistry for Developmental Stage, Prosthodontics, Periodontology and Endodontology, Team Care for Oral Health, Dentistry for Handicapped/Aged Persons, Dental Practice Administration etc.

Oral Health Education : Oral Health Education, Practice of Oral Health Behavior, Social Health Science, Communication Techniques, Medical Information Technology, Dental Education etc.

Oral Health Management (Oral Health Science Course) : Introduction of Oral Health Science, Practice of Oral Health Management, Practice of Oral Health Counseling, Dysphagia, Practice of Rehabilitation of Oral Function, Sports Dentistry / TMD etc

Oral Biomaterial Engineering (Oral Health Engineering Course) : Applied Biomaterial Engineering, Practice of Biomaterial Engineering, Introduction to Oral Health Engineering, Precision Casting, CAD System A and B, Medical Engineering A and B etc.

Medical Design and Engineering (Oral Health Engineering Course) :

Clinical Practice / Clinical Laboratory Work and

Graduation Research
"Yogo Teacher" (School Nurse) Program (Additional)

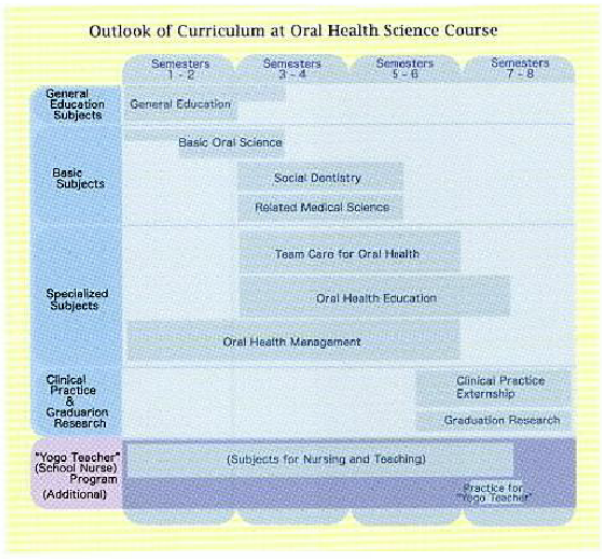

Fig. 1 The outlook of curriculum at Oral Health Science Course

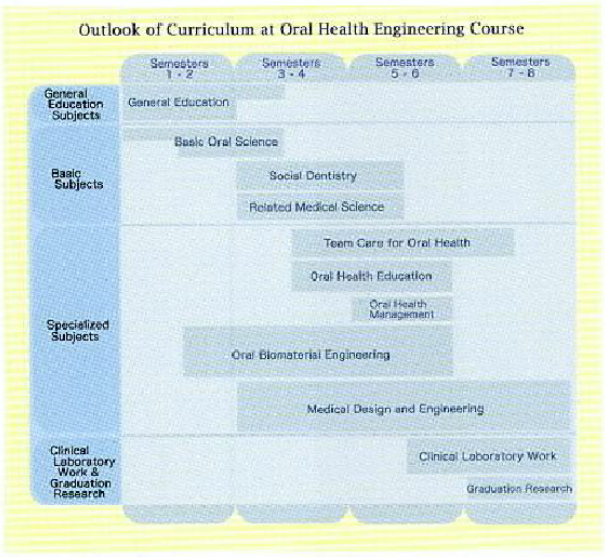

Fig. 2 The outlook of curriculum at Oral Health Engineering Course

3-1. Division of Oral Health Science

We have more than 140 dental hygienist schools in Japan, and 5 of them have 4-years Education system. Since the Minister of Health and Labor recently stipulated that the period of compulsory education for dental hygienist should be more than 3 years no later than 2010, most of them have been transferred into 3-years education.

The aim of the School of Oral Health Science is to promote oral health leading to healthy life of the people on all life-stages. The Division of Oral Health Science is to comprehensively study on oral health, to establish evidence-based medicine and raise people who can conduct world-wide research. This division will be dedicated to raise dental hygienists who will practice evidence--based medicine and promote oral health leading to healthy life of the people. To train "yogo teacher" (school nurse) for nursing in school who will push forward with management of school health, in particular, health education based on the concept of health promotion is also expected in order to raise people with mental and physical health. Furthermore, this division will be dedicated to train people doubly--qualified as dental hygienist and "yogo teacher" will exercise leadership in the field of oral health at school. In Fig. 3, the some examples of practice or lecture in our school are exhibited.

Fig. 3 Examples of the scene of practices in Division of Oral Health Science

3-2. Division of Oral Health Engineering

The division of Oral health engineering is the only one institute in Japan which possesses the four-year education for dental technicians. In deed, we have 64 dental technicians school in our country. One has the 3-years education for dental technician diploma course, and two of these are two-years college, and the remainder, 60 are the training schools (Fig. 4).

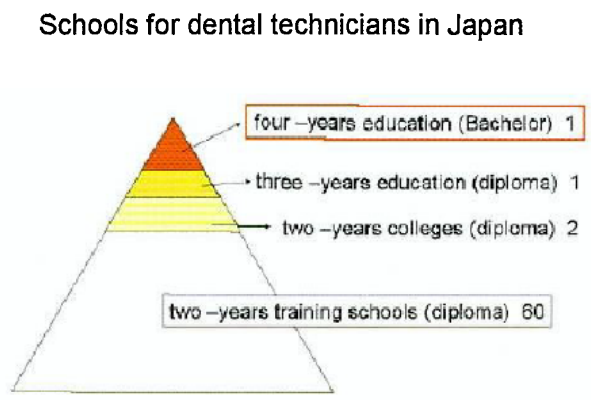

Fig. 4 In Japan, we have 64 dental technicians school and our division is only one institute to provide the 4-years education for the students.

In the dentistry, the biology-based or molecular-biology based research or clinical studies have been intensively developed, and most of these studies have been done by the dentists who have at least 6-years education, and most of them had additional 4-years education in their PhD course. In near future we will have the molecular-based or biology based therapies, such as regeneration of teeth, in the clinical dentistry. However, in the dental technicians school, they have been educated only the skill about traditional dentistry, i.e. how to make crowns or bridges, or dentures, which have not been altered in the century !! (Fig. 5).

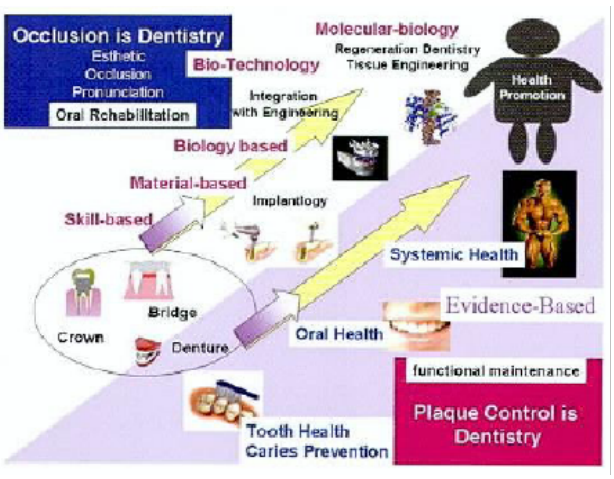

Fig. 5 There is a great gap between developed dental research and education of dental technicians school.

Hence, we should bridge the gap that exists between co-dental staffs and dentists or researcher in dental fields and cultivate human resources capable of doing the research or clinical works in the view point of biology or molecular biology and/or engineering.

The aims of the division are 1) to carry out the biological, and molecular biological studies based upon engineering, and 2) to promote the translational researches in the oral health engineering, in addition to the conventional dental technology. The curriculum encourages the person to open out the new fields and rustle in the whole world. Specifically, the division will turn out the unique dental technicians who develop the biomaterials and/or medical equipments, and exploit the computerized systems to design the medical devices based upon the biological and technological intelligence.

Figure 6 shows our curriculum, which is divided into 4 major categories, green shows the engineering related classes, orange shows biology-related one, yellow indicates advanced skill, and white is the conventional and traditional skill practice.

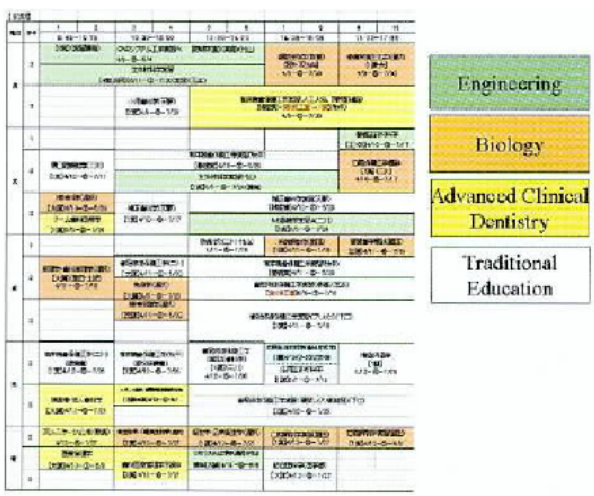

Fig. 6 An example of curriculum for our new education.

On current day, I will show you the classes or practices collaborated with industries and university. Please look forward to seeing the Characteristic Curriculum!

4. Extracurricular activities

Extracurricular activities produce significant educational benefits to encourage students to learn on their own and consider the human relationship. There

are two scientific "Club", called Hyper Technology Club (HiTec) and Bio Technology Club (BiTec) in the school. They took some awards that is "Front Runner Program Award" and "Dream Challenge Award" in Hiroshima University, respectively, and the students belong to the club enjoys the scientific investigations and/or activities.

4.1 Hyper Technology Club (HiTec)

HiTec (supervised by Prof. Murayama) was established in 2005, aims to learn high technology voluntarily and to try to apply the technology to dentistry. Most of the members are the undergraduate students belonging to Division of Oral Health Engineering. They have carried out the following activities.

1) Fabrication of physical maxillofacial models: As shown in Fig.7, they make 3D models from CT image data, and using the 3D models they fabricate physical models by rapid prototyping technology.

Fig. 7 Fabrication of physical maxillofacial models from CT image data

2) 3D scanning: As shown in Fig.8, they make 3D models, using 3D Scanner which uses laser to scan 3D form of a body.

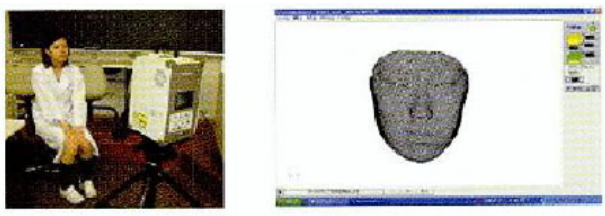

Fig. 8 3D scanning

3) Development of robots: They have made several robots using model robot kits to learn the mechanism of robots. Now they are trying to develop a micro robot, aiming to take part in Micro Robot Contest.

4.2 Bio-Technology Club (Bi-Tec)

Bi-Tec (supervised by Prof. Kurihara and Prof. Nikawa) was established in 2005, aims to enjoy the biology, microbiology and molecular-biology. The Club is comprised of nineteen undergraduate students belong to both the school of Dentistry and Oral Health Science. They have carried out the following activities.

1) Application of Probiotics in Oral Cavity
They isolates the probiotic bacteria, which shows antimicrobial activity against mutans streptococci or Candida albicans (Fig.9). Then identify them genomically (Fig.9), and try to make fermented milk to reduce the risk of dental caries.

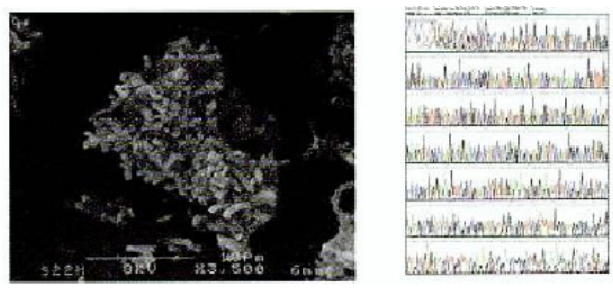

Fig. 9 An example of isolated probiotic bacteria and the results of genome analysis on the isolate.

2) Surface treatment to suppress the differentiation of pre-osteoclastic cell.
They examine the effects of immobilization of several kinds of peptide (Fig. 10) or growth factors on the titanium surface either on the growth of osteoblastic cells, or the differentiation of pre-osteoclastic cell (Fig. 10), to intend to develop a new implant system with short healing periods and good prognosis.

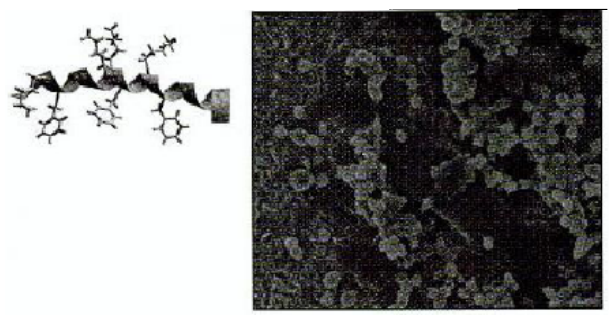

Fig. 10 An example of peptide and Osteoclastic cell

5. Conclusion

The oral health is the important factor to enhance the health and quality of life of all people. In Japan, the number of dental caries was decreased recently and the DMF of the 12 years old children showed 1.68 in 2006. But the our country's rapidly aging population and a declining birthrate need the prevention and treatment of periodontal diseases and the oral health care for old people. Therefore, the Cabinet Office has released "The New Frontier Strategy for Health" and recommend "The Power of the Healthy Tooth" to enhance the health of the nation in 2007,

The oral health science must summarize dentistry and provide better welfare and dental care that people need to live healthy. Now we are preparing to establish the postgraduate course which provides both advanced skills and most-advanced scientific researches.

Poster Session

■*Hiroshima University, Japan*

01-1

Minocycline, a Tetracycline Derivartive, Is Cytoprotective Against Excitotoxicity *Actinobacillus Actinomycetemcomitans* Serotype B by Inhibiting PARP-1, NFκB, Intracellular Calcium and Apoptosis of Gingival Epithelium

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BACKGROUND : Actinobacillus actinomycetemcomitans serotype b has long been associated with localized aggressive periodontitis,. Currently suggested that AA serotype b secreted protein toxin that inhibits the proliferation of wide variety of cell types. Eucariotic cells that are sensitive to the toxin are usually arrested at the G0/G1 or G2/M phase of the growth cycle through the action of a DNA-se I- like nuclease that causes double strand breaks in the host cell DNA. Gingival epithelial cell exquisitely sensitive to the toxin so that may lead to disruption of the protective barrier formed, facilitating invasion and perturbation of the underlying connective tissue. Minocycline, a semysinthetic tetracycline derivative un related to it's antimicrobial effect, protects against brain ischaemia, ischemic stroke, multiple sclerosis and parkinson's disease. In this study, we examined the ability of minocycline to inhibit apoptosis of gingival epithelium induced excitotoxin bacteria AA serotype b in mice.

OBJECTIVE : to investigated the cytoprotective effect of minocycline 100 nM against apoptosis in gingival epithelium induced toxin AA serotype b.

METHOD : Twenty eight adult mice strain Swiss Webster (balb C) were divided randomly into three groups : control group (group A), toxin group (group B) and application toxin and minocycline group (group C). The mice were sacrificed at 24 hours after application, and then the tissue sections of gingival epithelium were stained with Tunnel assay and imunohistochemistry method.

RESULT : Treatment with these toxin induced apoptosis of gingival epithelium was associated with DNA fragmentation, increased PARP-1 ,NFκB expression and followed by increased intracellular calcium. Minocycline 100nM significantly reduced apoptosis, PARP-1, NFκB expression and intracellular calcium ($p < 0,005$).

CONCLUSION : Minocycline 100 nM inhibit apoptosis by inhibiting PARP-1, NFκB and intracellular calcium of gingival epithelium. The improved function is associated enhanced cellular survival of gingival eipthelium after treatment with minocycline. **Minocycline 100 nanomolar** provides cytoprotection against excitotoxicity activated toxin bacteria AA serotype b, and **potential as new theurapetic agent to prevented localized aggressive periodontitis.**

Key words : Minocycline, PARP-1, NFκB, intracellular calcium, toxin AA serotype b

01-2

The Use of Piper Betle Linn Extract Tooth Paste and the Level of Mutans Streptococci in Children

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The aim of the study was to examine the level of Mutans Streptococci in saliva after the use of Piper Betle Linn Extract Tooth Paste in children. The samples were the students of Kendangsari Elementary School Surabaya Indonesia in range of age 10 to 12 years old, with good oral hygiene, DMFt 3-5, and no calculus. The samples were divided into experiment group (tooth paste + fluoride + piper betle linn) and control group (tooth paste + fluoride), with 10 subjects each. Prior to experiment both groups were trained with roll brushing method and 2 ml of saliva samples were collected. After tooth brushing using roll technique, the saliva of the experiment group and control group were re-collected. 0.22 ml of saliva samples of each group was cultured in 2 ml of BHI broth, and then 0.6 ml of the solution was taken and cultured in Mitis Salivarius Bacitracin, incubated anaerobically in 37℃ for 48 hours. The colony counting was done and statistically tests using Wilcoxon Signed Rank Test and Mann-Whitney Test. The result showed that level of Mutans Streptococci decreased significantly after tooth brushing using Piper Betle Linn Extract Tooth Paste compared with control group.

01-3

The Role of Gene Expression Patterns in Aggressive Periodontitis in Surabaya-Indonesia

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BACKGROUND : Aggressive periodontitis is a specific type of periodontitis with clearly identifiable clinical findings, rapid attachment loss and alveolar bone destruction. Genetic variations may also act as protective or risk factors for certain conditions, including periodontitis. This high familial aggregation of cases indicates that genetic factors are important in susceptibility Aggressive periodontitis, most genetic research in periodontitis has focused on gene polymorphisms that play roles in immunoregulation or metabolism, such as cytokines, cell-surface receptors, chemokines, enzymes and others that are related to antigen recognition. Familial aggregation reports have shown clustering within families.

OBJECTIVE : Localized Aggressive periodontitis is prevalent many adolescent and young adult in Surabaya. The prevalence increased from 9% in 1991 to 13% in 2003. These reports the role of gene expression pattern in aggressive periodontitis in Surabaya - Indonesia.

LITERATURE STUDY : Interleukin-1 (Interleukin-1a and interleukin-1b) plays an important role in periodontitis, two functionally similar molecules, have been recognized as central pro-inflammatory cytokines. However, these findings were not supported by other studies on interleukin-1 polymorphism in aggressive periodontitis in Caucasian African-American, North European, and Brazilian.b Polymorphisms within the interleukin-10 gene promoter appear to influence regulation of its expression. Association of the interleukin-10 gene with aggressive periodontitis, but no positive outcome was reported. The association between Fc gamma receptor gene polymorphisms and the risk for aggressive periodontitis have been investigated. Although the results varied among studies, it seems that the Fc gamma receptor- encoding gene is related to susceptibility to aggressive periodontitis. Results suggested that the genetic polymorphism at the Fc alpha receptor I ligand-binding site may be associated with susceptibility to aggressive periodontitis in Japanese individuals.

CONCLUSION : Identifying genes can therefore result in novel diagnostics for risk assessment, early detection and individualized treatment approaches, that contribute to the pathogenesis of periodontitis agresive therapeutic and scientific repercussions. Age limitations in diagnosing clinical phenotypes present unique difficulties in genetic analysis because periodontal information may be unavailable on the very young few family linkage studies of aggressive periodontitis have been carried out and the results are conflicting.

02-1

Effects of β-(1,4)-Acetylated Polymannose on Epithelial and Fibroblast Proliferation, Type I Collagen Expression and Wound Healing

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Wound healing comprised of 3 phases : inflammatory, proliferative and remodeling phases. Epithelial cells and fibroblasts play the important role in proliferative phase by proliferation and secrete several extracellular matrix proteins such as collagen.

OBJECTIVES : To investigate effects of β-(1,4)-acetylated polymannose, the polysaccharide extracted from Aloe vera gel, on proliferation of oral epithelium and fibroblasts, expression of type I collagen and its effect on oral wound healing in rats.

METHODS : Oral epithelial cells and fibroblasts were treated with different concentrations of β-(1,4)-acetylated polymannose (2.5, 5, 10, 20, 40, 80 and 160 nM) for 24 hours. Cell proliferation was determined by [^{3}H]-thymidine incorporation assay. The level of type I collagen expression were detected by ELISA. Punch biopsy wounds at hard palate of Sprague Dawley rats were used to evaluate effect of β-(1,4)-acetylated polymannose on oral wound healing. Animals were divided into 6 groups. In group 1, animals were topically treated with normal saline as negative control and group 2 were treated with Kenalog® as reference treatment. In group 3, animals were treated with plain CarbopolR, mucoadhesive polymer. In group 4, 5 and 6, animals received Carbopol® containing β-(1,4)-acetylated polymannose 50, 100 and 200 nM, respectively. All vehicles were applied daily and wound areas were observed at day 7.

RESULTS : β-(1,4)-acetylated polymannose (20 and 40 - 160 nM) significantly stimulated epithelial and fibroblast proliferation, respectively ($p<0.05$). In addition, β-(1,4)-acetylated polymannose at concentration 40-160 nM significantly enhanced type I collagen expression ($p<0.05$). The wound healing of animals received β-(1,4)-acetylated polymannose 50 nM were significantly better than the animals received Kenalog® and plain Carbopol® ($p<0.05$).

CONCLUSION : These findings suggest that β-(1,4)-acetylated polymannose plays a role in wound healing process, at least via the induction of epithelial and fibroblast proliferation and stimulation of type I collagen expression.

This study was supported by Thai Government Research Fund 2004-2006 and the 90th anniversary of Chulalongkorn University Fund (Ratchadphiseksomphot Endowment Fund) 2007.

02-2

Expression of Cytolethal Distending Toxin of *Aggregatibacter Actinomycetemcomitans* Causes DNA Damage and Induces Apoptotic Cell Death in Budding Yeast

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ABSTRACT

Aggregatibacter actinomycetemcomitans (Aa) is a bacterial species important in the pathogenesis of periodontal diseases. It possesses many virulence factors including the production of several toxins, one of which is Cytolethal Distending Toxin (CDT). AaCDT has been shown to cause G2 cell cycle arrest and apoptosis in many cell types, and thus may play a role in its pathogenicity. To characterize the mechanisms of CDT-induced cell death, we used the genetically tractable budding yeast, *Saccharomyces cerevisiae*, as a model system. We introduced the catalytic subunit CdtB, which has homology to DNase I, into yeast cells under the control of a galactose-inducible promoter. CdtB expression is toxic to wild-type yeast cells as shown by lower growth on galactose-containing medium. The toxicity is abolished by the mutation of the conserved DNase catalytic Histidine residue (H274A). This indicates that DNase activity is critical for CdtB toxicity and implies that CdtB induces cell death by causing DNA damage. To test this hypothesis, we expressed CdtB in yeast strains defective in DNA repair, such as *rad51Δ* and *rad53Δ* strains. As expected, these strains are hypersensitive to CdtB induction. In contrast, yeast strains with deletions of genes important in apoptotic cell death pathways show resistance to CdtB expression. This suggests that CdtB-induced yeast cell death is apoptotic and requires active participation of apoptotic genes, such as *YCA1*, the yeast metacaspase gene. In conclusion, our results suggest that CdtB expression causes DNA damage and induces apoptotic cell death in yeast. Highly conserved features of apoptosis and DNA repair pathways in yeast and mammalian cells ensure that this model system will be useful in further dissecting the mechanisms of CDT toxicity that is applicable to human cells.

03-1

p53 Gene Mutation and p53, MDM2, Ki67, MMP9 Expression in Oral Squamous Cell Carcinomas in Vietnamese Patients

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OBJECTIVES : To investigate p53 gene mutation and the pattern of *p53*, MDM2, Ki67, MMP9 expression in oral squamous cell carcinomas (SCC) in Vietnamese patients and to evaluate their prediction values.

METHODS : Immunohistochemical staining was performed in 110 oral SCC sections. PCR-SSCP and DNA sequencing were proceeded for analysis of exons 5-8 of *p53* gene in 18 cases.

RESULTS : A relatively high rate of mutations (44.4%) in exon 5, 7, 8 of *p53* gene was detected, notably in exon 8 and most of them were missense mutations. Protein overexpression was found in 75.5% cases for p53, 67.3% for Ki67, 56.4% for MMP9 and 36.4% for MDM2. p53 positive staining showed a significant association with high histologic grade, nodal metastasis. MDM2 positive staining was mostly found in low-grade tumors, whereas increased Ki67 expression was commonly observed in high-grade tumors. MMP9 overexpression was significantly correlated to radioresistance, poor survival, high histologic grade and nodal metastasis.

CONCLUSION : *p53* gene mutation and increased p53, MDM2, Ki67, MMP9 expression were often found in oral SCC in Vietnamese patients. Overexpression of p53, Ki67 and MMP9 was associated with poor prognostic factors in oral SCC.

03-2

Betel Chewing and Oral Mucosal Lesions in a Vietnamese Population Sample

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Betel quid chewing is a habit that was introduced in Vietnam milleniums ago and nowadays, it is still practiced in rural areas, mostly among elderly women (6.7% of female population). Clinical studies on oral cancer patients showed a relatively high prevalence and gender specific site distribution of oral cancer in females that could be attributed to this habit (63.3% female oral cancer patients were betel chewers). This study was conducted in a population sample living in the outskirts of Ho Chi Minh city where betel plants and areca palm trees are grown. The objectives of the study were to investigate the modalities of betel quid chewing and to determine the prevalence of oral mucosal lesions. Two groups, one of 152 chewers and the other of 137 non chewers, comparable in age and living conditions were included in the survey. Direct examination was conducted by 2 calibrated examiners and was followed by an interview on the betel chewing habit. Oral mucosal lesions were classified and recorded according to the WHO guide lines for oral mucosal lesions survey, 1995. The results of the survey showed that 98% of chewers were above 60 years old and 84.3% had had this habit for more than 20 years. The betel quid usually consist of a betel leaf, a piece of areca nut and slaked lime. Tobacco is a main ingredient of the betel quid (81.7%). The prevalence of oral mucosal lesions was significantly higher in chewers (80.4%) as compared to non chewers (37.2%) in cluding : betel chewer's mucosa (66%), oral submucous fibrosis (13%), lichen planus (5.2%) and leukoplakia (3.9%). In non chewers, all other lesions were not detected apart from lichen planus which was observed in 1.5% cases. It is concluded that oral mucosal lesions were significantly more prevalent in the betel quid chewing population. Therefore issues related to the prevention and management of such lesions should be addressed in health care programs of this particular region.

04-1

Current Developments in Candida Biofilm Proteomics, Cellular Imaging and Drug Resistance

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BACKGROUND : *Candida* infections primarily begin with surface adherence of cells leading to the formation of surface attached communities known as biofilms. The major clinical relevance of *Candida* biofilms is their high resistance to antifungals.

OBJECTIVES : The mechanisms underlying *Candida* biofilm formation and their resistance to antifungals are poorly understood. In this study, we used a proteomic based approach to characterize the protein markers that are differentially expressed in *Candida* biofilms in comparison to planktonic counterparts.

EXPERIMENTAL METHOD : Both reference and wild type strains of C. *albicans* were used. *Candida* biofilms and age matched planktonic (free floating) forms were obtained in parallel. Antifungal tests were done for commonly used antifungals, nystatin, amphotericin-B, ketaconazole, 5-FC and caspofungin. Afterwards *Candida* biofilm and planktonic protein were extracted according to the standard protocol and subjected to two-dimensional gel electrophoresis. Comparative gel analysis was done for elucidation of up and down regulated proteins in Candida planktonic and biofilm proteome. Protein identities were deduced using Tandem Mass Spectrometry and bioinformatics algorithms. Furthermore, transcriptomic regulation of selected genes was analyzed by RT-PCR. These proteomic experiments were coupled with Scanning Electron microcopy (SEM) and Confocal Scanning Laser Microscopy (CSLM) imaging of *Candida* biofilms.

RESULTS : *Candida* biofilms had higher antifungal resistance for all five tested antifungals compared with planktonic mode. SEM and CSLM showed biofilm architecture of a structured community with varying morphotypes, embedded in a voluminous matrix of extra cellular polysaccharides. Proteins that were present in preponderance were antioxidants such as alkyl hydroperoxide reductase, thioredoxin peroxidase and thioredoxin. In addition, a drug target for echinocandin was significantly up regulated in *Candida* biofilms. Transcriptomic analysis revealed that some of these genes were up regulated even at transcriptomic level.

CONCLUSION : Taken together, these data imply that the increased antioxidants may contribute to the higher antifungal resistance seen in *Candida* biofilms.

ACKNOWLEDGEMENT : Hong Kong Research Grants Council, RGC No. HKU 7624/06M

05-1

Comparison of Dento-Skeletal Conditions of Orthodontics Patients With and Without Unilateral Complete Clefts of Primary and Secondary Palates

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BACKGROUND AND RATIONALE : Dento-skeletal growth and development of patients with cleft lips and palate are affected by genetic factor, initial surgical operations, and orthodontic treatment. Therefore, the present study involves cephalometric analysis of patients with unilateral complete cleft lip and palate in order to clarify their dento-skeletal morphology and to evaluate their treatment outcomes following primary surgical repair.

PURPOSE : To quantify and compare occlusal and skeletal jaw relationships among children with and without congenital unilateral complete clefts of primary and secondary palate

METHODS : The study concerned dento-skeletal conditions of 72 children (mean age 11.3 years) born with unilateral complete clefts of primary and secondary palates (UCLP) in Thailand who received primary repair surgery from Khon Kaen University and seventy-two children (mean age 11.8 years) orthodontically treated Non-cleft patients from the same population, matched for age and gender. The control group comprised one hundred and seventy-three children (mean age 13.09 years) with Angle's Class I and acceptable facial profile. Cephalometric analysis was used to determine dento-skeletal conditions. All measurements in each group were compared by use of One-way ANOVA and multiple comparisons test (Dunnett T3)

RESULTS : In comparing UCLP with Non-cleft and normal group, anterior cranial base length was significantly smaller, more retrusive position in UCLP than in Non-cleft and normal group, maxillo-mandibular position(ANB) were significant more Skeletal Class III compared with Non-cleft group.($p < 0.05$)

CONCLUSION : The subjects with UCLP had dento-skeletal conditions that differed significantly from non-cleft patients with malocclusion and a normal group. It is further speculated that the Thai patients in this study and those from other Asian studies have more Class III malocclusion and skeletal Class III pattern among the cleft subjects than among equivalent Caucasian groups. This could be attributed at least in part to combined effects of the primary surgery and racial differences.

05-2

Knowledge and Attitude of Dental Students towards Student-Centered Learning at the Faculty of Dentistry, Khon Kaen University

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OBJECTIVES : To evaluate the knowledge and attitude towards student-centered learning (SCL) among first- to sixth-year dental students enrolled in the Faculty of Dentistry, Khon Kaen University during the first trimester of 2005.

METHODS : Data were collected by a self-administered questionnaire (Cronbach's alpha=0.97) and presented with descriptive statistics.

RESULTS : Two hundred and eighty-eight students (96.6%) responded to the questionnaire. Students had a mean SCL knowledge score of 11.9 (SD=2.1) (out of 16). Females had higher knowledge scores than males. Students with better cumulative grade point average (GPAX) scored higher than did those with lower GPAX. Majority (81.3%) of the students agreed that SCL was a useful teaching-learning approach. However, only 42.7 percent indicated they liked SCL, in comparison to 57.0 percent who disliked it. Moreover, most of the students (72.6%) responded that SCL was not an appropriate method to use in the dental school. When asked to choose appropriate approach for each subject that they had previously taken, students ranked lecture method as best-suited for 19 subjects and direct instruction method for 10 subjects. Direct instruction method was also ranked as second-best for 24 subjects, and case study method as third-best for 28 subjects. The largest proportion of the students (36.8%) liked the direct instruction method most and 29.2 percent considered it as the most applicable method, while 36.8 percent thought that the lecture method helped them best in understanding the lessons.

CONCLUSION : Dental students had good knowledge about SCL, while the attitudes were somewhat mixed. Although students appreciated its usefulness, the majority disliked the SCL approach and indicated that it was not suitable for the dental school curricula. Teachers and learners should work together to develop educational methods that effectively help them achieve academic goals and satisfying learning experience.

06-1

Expression of Ki-67, p53, MDM-2 and Bcl-2 at the Tumour Invasive Front in Oral Squamous Cell Carcinoma

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BACKGROUND AND RATIONALE : Oral squamous cell carcinoma (OSCC) is the 6th most common cancer worldwide. While treatment modalities have improved over the years, the survival rate of OSCC patients remained low. Recent studies had shown that assessing the activities of several markers at the tumour invasive front might prove to be useful in providing essential information regarding the tumour's aggressiveness.

STUDY OBJECTIVES : Current study aimed at describing the immunoexpression of several markers such as Ki-67, p53, MDM-2 and Bcl-2 at the tumour invasive front in OSCC and investigating the relationship of the immunoexpression of these markers with demographic and clinicopathological characteristics in particular the pattern of invasion.

DESIGN AND EXPERIMENTAL METHODS USED : Forty five formalin fixed, paraffin embedded OSCC samples from Diagnostic Laboratory, UM, were included in the study. Demographic and clinicopathological characteristics were recorded. Immunohistoche- mistry with antigen retrieval method was used to assess the expression of Ki-67, p53, MDM-2 and Bcl-2. Pearson's chi-square and Fisher's exact test were employed for statistical analysis.

RESULTS : The study comprised of 25 male (55.6%) and 20 (44.4%) female subjects with a mean age of 59.0 year (±11.90). Most subjects were in Stage III (25.7%) and IV (42.86%). A high number of samples showed non cohesive pattern of invasion (68.9%) than cohesive (31.1%). Twenty eight (62.2%) samples showed positive Ki-67 immunostaining, 34 (75.6%) were positive for p53, 44 (97.8%) were positive for MDM-2 and only two (4.4%) were positive for BCl-2 immunostaining. All positive staining were observed at the tumour invasive front. None of the overexpression was significantly related to any parameters investigated.

CONCLUSION : This study revealed a high number of cases expressing Ki-67, p53 and MDM-2 protein except for Bcl-2 at the tumour invasive front in OSCC.

07-1

Application of MR Virtual Endoscopy as a Presurgical Procedure before Sialendoscopy

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OBJECTIVE : To investigate the feasibility of clinical application of MR virtual endoscopy as a presurgical procedure before sialendoscopy, and to evaluate its value in the diagnosis of obstructive salivary gland diseases and preoperative visualization of endoluminal views.

STUDY DESIGN : This study presents our initial experience to use MR virtual endoscopy for the presurgical visualization of salivary duct lumen and ductal pathologies in comparison to the sialendoscopic findings in a feasibility study.

METHODS : Six consecutive patients with suspected obstructive salivary gland diseases underwent MR sialography with a three-dimensional fast imaging employing steady-state acquisition. The 3D MR data were transferred to an independent workstation and were post-processed with navigator software to generate three-dimensional reconstruction and virtual endoscopic images. The fly-through mode was used to imitate the sialendoscopic exploratory procedure. Then the patients underwent sialendoscopy and the endoscopic findings were compared with the preoperative virtual endoscopic images.

RESULTS : The MR data acquisition and post-processing protocol were feasible. The virtual endoscopy created clear endoluminal views of salivary duct and the ductal pathologies. The diagnoses were all confirmed by surgical sialendoscopy. The virtual endoscopic images showed close resemblance to the sialendoscopic findings.

CONCLUSIONS : MR virtual endoscopy is an effective and noninvasive diagnostic method for evaluating the endoluminal anatomy and pathologies of salivary duct. The clinical application of MR virtual endoscopy as a presurgical procedure before sialendoscopy is a valuable and promising approach which can provide surgeons useful morphological and pathological information.

07-2

Efficacy of ProTaper Universal Nickel-Titanium Rotary System for Gutta-Percha Removal in Endodontic Retreatment

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Contents of Abstracts

INTRODUCTION : The main goal of nonsurgical endodontic retreatment is the complete removal of the preexisting root canal filling materials.

OBJECTIVE : To evaluate ProTaper Universal nickel-titanium rotary system for gutta-percha removal during root canal retreatment.

MATERIALS AND METHODS : Sixty extracted human anterior teeth were randomly assigned to three groups of 20 specimens each. The canals were enlarged and obturated with lateral condensation technique. Retreatment for group A was begun with ProTaper Universal rotary retreatment system, in which retreatment files were used to reach the original working length. Canal refining was accomplished with ProTaper nickel-titanium rotary shapig and finishing files. In group B, filling materials was removed using Gates Glidden drills, and Hedstrom files with chloroform. ProTaper rotary shaping and finishing files were also used to finish re-preparation of root canals. For group C, the same method as group B was used for gutta-percha removal. Canal reshaping was accomplished with stainless steel K-flex files. The following parameters were evaluated : time for reaching the original working length, time for gutta-percha removal, total time, debris extruded apically. After clearing the teeth, the area of remaining obturation material was measured and compared statistically from 2 directions.

RESULTS : ProTaper Universal rotary technique in group A worked significantly faster than the techniques used in groups B and C ($p<0.05$). Retreatment in groups B and C resulted in a higher amount of debris extrusion compared with group A, with a significant difference between groups A and C ($p<0.05$). As for the canal cleanliness, group A had a smaller percentage of areas covered by gutta-percha and sealer than groups B and C, with the difference between groups A and C being significant ($p< 0.05$).

CONCLUSION : The ProTaper Universal rotary system was proved to be an efficient and time-saving device for gutta-percha removal in endodontic retreatment.

08-1

Correction of a Class II Division I Malocclusion Using a Modern Bionator Appliance

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BACKGROUND AND RATIONALE : The bionator appliance was developed for class II corrections with mandible retrusion, but there are some limitations due to lower incisor flaring or crowding. In addition, much research has shown there to be no change in the forward growth of the maxilla. To overcome these shortcomings, some modifications were developed in this new modern bionator.

HYPOTHESIS OR STUDY OBJECTIVE : The aim of this study was to present a modern bionator appliance with [high pull?] head gear for treatment of cases of class II malocclusion with mild dental crowding. The bionator not only can correct mildly crowded teeth, but can also control maxillary growth and advance the mandible to achieve a class I [correction/status?] and facial harmony.

DESIGN AND EXPERIMENTAL METHODS USED : Two patients with class II division I malocclusions during Hellmann's dental age IIIB stage were selected for bionator treatment. They were instructed to wear the bionator and the high pull headgear for at least 10 h a day. The design of the modern bionator includes labial wires and resin caps at the upper and lower anterior segments. By adjusting the labial bow loops, the anterior teeth can be retracted and uprighted. In the buccal segment, there are occlusal resin wedges to force the posterior teeth to tip distally. With the addition of a mandibular lingual expansion screw, the dentoalveolar arch can be expanded, making the teeth less crowded. Active treatment time is about 1.5 years.

ESSENTIAL RESULTS : Cephalometric analysis showed that the SNA and ANB angles decreased and the SNB angle increased in these cases after treatment. Both the upper and lower incisors were uprighted and the molars were tipped back; crowding was also relieved. The treatment results for these two patients were very acceptable.

CONCLUSIONS : From these two case reports, we show that a modern bionator is effective in class II division I treatment for both skeletal and dentoalveolar correction without using fixed appliances.

09-1

Clinical and Histological Evaluation of RBM-Surfaced Implants Placed Immediately in Fresh Extraction Sockets of Beagle Dogs

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BACKGROUND : Numerous studies have been conducted to enhance surface geometry of implant. Resorbable blast media(RBM)-surfaced implant was specifically developed to provide uniform roughness and enhanced surface for osseointegration of titanium.

Nowadays, a variety of domestic implants are being developed. However, the data related to surface characteristics of these materials are quite insufficient.

PYRPOSE : The aim of this study was to compare the reaction of peri-implant tissues to three kinds of RBM-surfaced implants placed immediately in extraction sockets of beagle dogs.

Materials and method : Three kinds of RBM-surfaced implants were randomly inserted into seven beagle dogs ; Lifecore (4.1 mm * 8 mm STAGE-1®, Lifecore, U.S.A.), Avana (4.0 mm * 8.5 mm SS-III®, OSSTEM , Korea.) and Dio (4.0 mm * 8 mm, IFI® DIO, Korea). Total of 21 implants were used. Clinical and histological examinations were performed at 6 weeks and 12 weeks after placement.

RESULTS : All the inserted site showed normal healing patterns without failure and inflammation. Periotest values (PTV) for clinical evaluation were not significantly different among the implants at both 6 and 12 weeks ($p<0.05$).

Histologically, bone to implant contact ratios (BIC) were 42.4±17.3% for Lifecore, 32.0±11.1% for Avana, and 34.9±20.3% for Dio at 6 weeks. At 12 weeks, BIC were 58.5±5.1%, 61.9±6.1%, and 57.5±6.0% in the same order. There were no significant differences among the implants at both 6 and 12 weeks. ($p<0.05$).

CONCLUSION : From the results of clinical and histological evaluation, 2 kinds of domestic implants were comparable to Lifecore implant in terms of stability of implant and degree of osseointegration.

10-1

Effect of Different Time Periods of Vital Bleaching on Flexural Strength of Bovine Enamel and Dentin Complex

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BACKGROUND AND RATIONALE : Vital bleaching needs some materials which should be beneficial and lead to the least tooth structural damage. These days patients show more tendency for bleaching their colored teeth but despite a lot of researches which have been done about vital bleaching, the principal issue that hadn't been investigated was the effect of vital bleaching agents on the flexural strength of the tooth.

OBJECTIVE : The aim of this in vitro study was to evaluate the effect of vital bleaching agents on the flexural strength of enamel and dentin complex in different time periods.

Methods and Materials : 100 sound bovine teeth were selected. Blocks (2×3×8 mm) from the middle portion of the facial surfaces of each crown were sectioned from the teeth. The dentinal part of the teeth was covered by wax.
Specimens were randomly divided into five groups (n=20) based on the time period of vital bleaching. Group 1 comprised the control group kept in artificial saliva. The experimental groups subjected to immersion in 20% carbamide peroxide, Opalescence, for two, four, six and eight weeks, respectively (eight hours daily). Mechanical testing was performed 24 hours after the last treatment using an Instron Universal Testing Machine with a crosshead speed of 0.5 mm/min. Data was gathered and ANOVA and Tukey test showed the statistically significant differences(α =0.05).

RESULTS : Statistically significant difference in flexural strength was noted among the groups. A statistically significant difference was observed between Group 2 (two week bleach) and the control (P<0.05).There was no significant difference among other groups. . In this context two hypotheses might be considered; first it could be that remineralization might have happened due to salivary action which could have compensated the initial decrease in the flexural strength of the specimens. Another explanation of the observed results might be the softening of the tooth structure due to the reduction of fracture toughness which has caused an increasing resistance of the specimens to fracture at 4, 6 or 8 weeks periods.

CONCLUSION : Application of 20% Carbamide Peroxide for two weeks provided significant decreases in FS of bovine enamel and dentin complex. Similar decreases were not observed among the other groups.

11-1

Effect of Radiation on Oral Cancer Cell-Osteoblast Interactions

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BACKGROUND : This work was to evaluate the effect produced by conditioned medium from oral squamous carcinoma cell (HSC3) culture on osteoblast (MC3T3-E1) osteoclastogenesis related cytokines.

OBJECTIVES : The aim of this study was elucidate the effect of carbon-ion irradiation on oral cancer cell bone invasion and cancer-induced osteoclastogenesis and related cytokines.

METHODS : HSC3 was cultured to confluence, and then exposed to 0, 2, 4, or 6 Gy of carbon-ion irradiation. MC3T3-E1 was cultured for 6 days after adding conditioned media derived from HSC3 cell cultures. The expression of parathyroid hormone related protein (PTHrP) mRNA in HSC3 and osteoclastogenesis related cytokines mRNA in MC3T3-E1 was measured using real-time PCR.

RESULTS : Carbon-ion irradiation suppressed the expression of PTHrP in HSC3 in a dose-dependent manner. Osteoblasts treated with conditioned media from carbon-irradiated cancer cells were significantly suppressed expression of osteoclastogenesis related cytokines mRNA between 70.94% and 20.94% depending on irradiated dose.

CONCLUSIONS : Carbon-ion irradiation prevented osteoclasts development, and PTHrP was a factor predicting bone invasion by oral cancer. The results suggest that carbon-ion irradiation may prevent bone invasion in oral squamous cell carcinoma.

11-2

Regeneration of Bone by Synthetic Peptide of Ameloblastin

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We previously found that ameloblastin, one of enamel matrix proteins, plays important roles in differentiation of periodontal ligament cells. It was recently reported that ameloblastin is related to craniofacial bone development in rats. As N-terminal of ameloblastin (AMB-N) is well conserved in different species, we examined regeneration effects of synthetic AMB-N peptide on bone both *in vitro* and *in vivo* models. First, we examined effects of AMB-N peptide on differentiation of osteoblasts. The peptide increased expression of alkaline phosphatase (ALP) and bone sialoprotein (BSP) mRNA, ALPase activity and minerarization in MC3T3-E1 cells. Moreover, enhanced ALPase activity by AMB-N peptide was abolished by addition of an ameloblastin antibody. Next, we examined effects of AMB-N peptide on bone regeneration in vivo by using a rat cranial bone defect model. After 4 weeks of surgery, AMB-N peptide treated calvarial bone defects were nearly completely filled with newly formed bone tissues, while newly formed bone tissues did not fill in control bone defects. Moreover, to examine the mechanism of mineralization by AMB-N peptide, we cloned 48 bp of N-terminal ameloblastin sequence into pFLAG-CMV3 with extracellular secretion sequence and pFLAG-CMV4 without extracellular secretion sequence. Then we obtained AMB-N expressiong and secreting cells (pFLAG -CMV3-AMB-N) and AMB-N expressing and non-secreting cells (pFLAG-CMV4-AMB-N). pFLAG-CMV3-AMB-N showed increased ALP and BSP mRNA expression, ALPase activity and minerarization, while pFLAG-CMV4-AMB-N did not. These findings suggest that AMB-N peptide may enhance mineralization mediated by the binding with unknown receptor on the cell surface. In summary, AMB-N peptide enhanced mineralization of osteoblasts both *in vitro* and in vivo. We suggest that AMB-N peptide can be used for bone regeneration.

11-3

Effect of Brain-Derived Neurotrophic Factor in Cementoblast Like Cells

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Brain-derived neurotrophic factor (BDNF) is known to play a role in proliferation and differentiation in various types of cells. We have reported that BDNF promotes the periodontal tissue regeneration in dog studies. BDNF was especially effective for cementum regeneration. Since the functional periodontium is structured with cementum, alveolar bone, and connective tissue fibers inserted into these hard tissues, the reconstruction of cementum on denuded dentin surface is a key to regenerate periodontal tissue with the functional periodontium. In the present study, in order to elucidate the regulatory mechanism of BDNF on cementoblasts functions, we examined the mRNA expression of the bone/cementum-related proteins (alkaline phosphatase [ALPase], osteopontin [OPN] and bone morphogenetic protein [BMP-2] and their signaling pathway after BDNF stimulation in cultures of human cementoblast-like (HCEM) cells. HCEM cells were immortalized by transfection of *hTERT* gene (Kitagawa et al. , 2006). The mRNA expressions were determined by real-time PCR. BDNF (20 ng/ml) caused a 2.9-fold increase in ALPase mRNA expression, a 2.2-fold increase in OPN mRNA expression and a 3.2-fold in BMP-2 mRNA expression in HCEM cells ($p < 0.01$, ANOVA). siRNA of trkB, a high affinity receptor of BDNF, siRNA of Elk-1, a downstream target of ERK1/2, and PD98059, an ERK inhibitor, abolished the increase in the mRNA levels ($p < 0.01$, *t*-test). BDNF increased the levels of phosphorylated ERK1/2 and Elk-1. siRNA of trkB and PD98059 suppressed the phosphorylation of ERK1/2 and Elk-1. Furthermore, BDNF increased the levels of phosphorylated c-Raf which activates the ERKs signaling pathway. These findings suggest that BDNF induces mRNA expression of ALPase, OPN and BMP-2 through a trkB-c-Raf-ERK1/2-Elk-1 signaling pathway in HCEM cells.

11-4

A Trial Appliance of Tailor Made Tissue Engineered Bone for Alveolar Ridge Augmentation

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BACKGROUND : Thin alveolar ridge needs augmentation by bone graft matrices for dental implant placement or denture stabilization. Autologous bone transplantation is used for the gold standard for ridge augmentation; however several disadvantages are included for its usage. Various artificial scaffolds has been examined for ridge augmentation, however a certain methods has not been established. Mesenchymal stem cells (MSC) can easily be obtained by bone marrow aspiration. Usage of MSC combined with artificial scaffolds might be useful and reliable tool for ridge augmentation.

OBJECTIVE : The objective of this study was to grope a method for alveolar ridge augmentation by minimally invasive surgery.

EXPERIMENTAL METHODS : A female beagle dog (13 month after birth) was used. Iliac bone marrow cells were aspirated and isolated MSC were maintained in ordinal medium. Bilateral lower premolars were extracted 8 months before transplantation. Beta-Tricalcium Phosphate (b-TCP; OSferion) blocks (50x30 x10 mm) were trimmed by sterilized drill to fit the edentulous alveolar ridge, and the block was immersed in $2x10^7$ MSC cells/5 ml medium. The MSC/b-TCP complex was cultured in bone inductive medium for 2 weeks. The calcified MSC/b-TCP complex was fixed to the right side of the edentulous ridge using titanium micro screw. b-TCP without MSC was fixed to the left side as a control. One month after transplantation, transplanted site of the jaws were taken out, decalcified, cut in thin specimen, H&E stained, then observed by microscope. The study was submitted according to the Ethical Committee for animal research, Hiroshima University.

RESULTS : Control side of the explant was exposed out of the mucosa after 2 weeks, so the explant was removed. Right side of the explant was stabilized well, and osteoid structure was observed in b-TCP.

CONCLUSION : Tailor made artificial bone made by b-TCP and MSC might be useful for alveolar ridge augmentation.

11-5

Influence of Elevation of GGT by Cholestatic Liver Disease on Bone and LPS Induced Alveolar Bone Destruction

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BACKGROUND AND STUDY OBJECT : It is well known that elevation of serum γ-gulutamyl transpeptidase (GGT) level is observed in patients with cholestatic liver diseases. Recently, we identified GGT as a novel bone resorbing factor. Furthermore, we demonstrated that topically applied GGT to rat gingival sulcus induced alveolar bone resorption. Theses findings indicate a possibility that the elevated serum GGT in patient may be responsible for reduced bone mass through activation of osteoclastogenesis. However, little is known about relationship between elevated serum GGT level and reduced bone mass. The aim of this study was to analyze effects of elevated serum GGT on bone quality and LPS-induced alveolar bone destruction using rat bile duct ligation (BDL) model.

MATERIAL AND METHODS : Seven-week-old Wistar strain male rats were divided into two groups subjected with BDL or Sham operation (Control). After 1 and 2 weeks, we measured serum GGT level and bone density of lower body (Dual Energy X-Ray Absoptimetry). Number of osteoclast and % bone mass in the femur and alveolar bone were histomorphometrically analyzed. Furthermore, 5 mg/mL *E. coli* LPS was topically applied to gingival sulcus of BDL and Control animals at 11 days after each operation to induce alveolar destruction. Number of osteoclasts appeared along the alveolar bone margin was counted at 3 days after LPS application.

RESULTS : In BDL group, marked elevation of serum GGT level was evident. Decrease in bone mineral density, loss of bone mass and increase of osteoclasts were induced associated with elevation of serum GGT level. In BDL animals with elevation of serum GGT level, osteoclast formation caused by LPS application was markedly promoted than Control animals.

CONCLUSION : These findings suggested that the elevated of serum GGT level in patients with cholestatic liver disease may be a potent risk factor of osteoporosis and periodontal disease.

11-6

Skp2 Expression Is Associated with Down-Regulation of p27 Protein and Cell Proliferation in Salivary Adenoid Cystic Carcinoma

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Adenoid cystic carcinoma (ACC) is a malignant salivary gland tumor, which shows frequent recurrence and metastasis, ultimately with a poor outcome. We previously demonstrated that p27 down-regulation is frequently found and is due to an enhancement of its degradation in ACC. In this study, we transfected nondegradable p27 mutant (T187A) and wild-type gene into ACC cell line. Transfection of T187A mutant gene was more effective on inhibition of cell growth of ACC cells, suggesting that aberration of p27 degradation may be present in ACC. As F-box protein S-phase kinase-associated protein 2 (Skp2), which is necessary for ubiquitin-mediated degradation of p27, is involved in p27 down-regulation in various cancers, we examined the Skp2 expression and its association with p27 expression in 50 ACC cases. We found Skp2 expression in 36% of ACC cases and inverse association between the expression of Skp2 and p27. Moreover, Skp2 small interfering ribonucleic acid (siRNA) transfection decreased Skp2 protein and accumulation of p27 protein and inhibited the cell growth of ACC cells in vitro. These findings, overall, suggest that Skp2 may play an important role in ACC development through the down-regulation of p27 and that Skp2 siRNA can be a novel modality of cancer gene therapy for suppression of p27 down-regulation in ACC.

11-7

Effects of Sex Hormone and TGF-β1 on Bone Growth in Newborn Mice

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It is well known that sex hormone exerts substantial influences on bone metabolism. Meanwhile, Transforming growth factor-beta1 (TGF-β1) is widely distributed in adult bony tissues, is an important regulator of bone metabolism. The purpose of this study was to investigate the effects of sex hormone and TGF-β1 on bone growth and metabolism in newborn mice. Orchiectomy (ORX) and Ovariectomy (OVX) were performed for five-day-old C57BL/6J mice and then 17-b estradiol (E_2) and 5α-dihydrotestosterone (DHT) were given daily immediately after surgery. At 1, 4, 8 weeks after surgery, these animals were sacrified for morphometric and immunohistochemical analysis and blood was taken for the quantification of TGF-β1 in serum. Furthermore, the mandible was resected immediately after birth and subjected to organ culture with anti-hTGF-β1 for twelve days.

Femure and mandibular growth were reduced in the ORX and OVX groups, however, growth deficiency was not found in the ORX and OVX groups with E_2 injection until 4 weeks after surgery. The expression of TGF-β1 in cartilage layers were substantially lower in the ORX and OVX groups. The concentration of TGF-β1 in serum were significantly smaller in the ORX and OVX groups than in the control groups. These phenomena were corrected by E_2 injection, but not by DHT injection in both sexes. In organ culture, mandibular growth was reduced significantly by anti-hTGF-β1 antibody. These results highly emphasize that estrogen deficiency causes the reduction of TGF-β1 in serum, which is speculated to induce bone growth disturbances. It is shown that sex hormone has an indirect pathway via TGF-β1 to osteoclast and chondrocyte as well as the direct effects.

11-8

Prostaglandin E_2 Inhibits Mineralization and Enhances Cementoblast-Mediated Degradation and Cementoclastogenesis Mainly via EP4 Pathway

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BACKGROUND AND RATIONALE : ProstaglandinE_2 (PGE_2) plays an important role in pathogenesis of periodontal disease and modulating bone metabolism via osteoblasts through PGE receptor subtypes (EPs). Cementoblasts share many characteristics with osteoblasts. There is a possibility that PGE_2-EPs pathway also regulates cementum metabolism. However, little is known about the effects of PGE_2 and EPs on functions of cementoblasts.

STUDY OBJECTIVE : The aims of this study were to examine the roles of PGE_2 and EPs on functions of cementoblasts, including mineralization and cementoblast-mediated Degradation and cementoclastogenesis.

DESIGN AND EXPERIMENTAL METHODS : OCCM-30 (a mouse cementoblast cell line; kindly provided from Prof. Somerman) cells were used in this study. Effects of PGE_2 and EP agonists on mineralized nodule formation (alizarin red S staining) and alkaline phosphatase (ALP) activity (Bessey-Lowry enzymologic method) were examined. Effects of PGE_2-EPs pathway on expression levels of bone sialoprotein (BSP), osteocalcin (OCN), matrix metalloproteinase (MMP)-13, receptor activator of NFkB ligand (RANKL), osteoprotegerin (OPG) and IL-6 were examined by real-time RT-PCR or ELISA. A co-culture system of cementoblasts and bone marrow cells was used to determine the ability of PGE_2 to promote the formation of TRAP-positive cells. Based on the in vitro results, effects of orally administrated EP4 antagonist on periodontal tissue destruction was evaluated with in vivo models.

ESSENTIAL RESULTS : PGE_2 and EP4 agonist caused down-regulation of mineralized nodule formation and ALP activity in OCCM-30. BSP and OCN mRNA expressions were suppressed and MMP-13 mRNA expression was stimulated via PGE_2-EP4 pathway in OCCM-30. PGE_2-EP4 pathway up-regulated RANKL and IL-6 levels, and down-regulated OPG level. EP4 antagonist reduced LPS/pressure induced of osteoclasts/cementoclasts formation in vivo periodontal tissue.

CONCLUSIONS : PGE_2-EP4 pathway may regulate cementum destruction through down-regulation of mineralization ability of cementoblasts and up-regulation of cementoblast mediated degradation and cementoclastogenesis. EP4 antagonist may be expected as a new periodontal therapeutic drug.

11-9

Saliva Secretion Is Stimulated by Tongue Exercise

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BACKGROUND AND OBJECTIVE : Chewing is well known to stimulate saliva secretion. However, once saliva secretion is impaired, concomitant chewing difficulty exaggerates the situation, especially for elderly people with removable prostheses. If tongue exercise can stimulate saliva secretion, elderly people could avoid disuse atrophy of saliva function in spite of chewing difficulty. For the present study we standardized tongue exercise and see its stimulating effect on saliva secretion.

METHODS : Fourteen male subjects (23 to 31 years of age) participated. All of them were keeping antagonistic occlusal contacts enable to be estimated as A1 by The Eichner's Index. For standardizing tongue exercise, our prototyped portable device for tongue pressure measurement was utilized. Subjects compress the intra-oral balloon section of the device onto their palate at the pressure of 30 kPa cyclically for 1 s with 1 s intervals for 5 minutes. Saliva secreted by this exercise was collected and measured it by weight and compared with stimulatory secretion by chewing 1.5 g of tasteless gum base and rest state secretion.

RESULTS : Mean ± S.D. of weight of collected saliva with tongue exercise were 0.79 ± 0.51 g/min, while 2.09 ± 0.70 g/min for gum base chewing and 0.52 ± 0.36 g for rest state secretion. More amount of saliva was secreted in response to tongue exercise than rest state secretion ($P<0.05$, paired t-test), but gum base chewing was more effective than the others ($P<0.01$).

CONCLUSION : Saliva secretion was stimulated by tongue exercise, though the amount of increase was less than that stimulated by gum-base chewing. Effect of continuous tongue exercise on saliva secretion could be the next concern.

11-10

Expression of Survivin and Aurora-B in Oral Cancer: Predictive Factor for Oral Squamous Cell Carcinoma

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Survivin, one of the inhibitors of apoptosis gene family, is strongly expressed in cancer cells and its overexpression in cytoplasm is well known to be associated with poor outcome in various cancers including oral squamous cell carcinoma (OSCC). Survivin has also defined as one of the chromosomal passenger proteins (CPPs) and concerts other CPPs such as INCENP, TD-60 and Aurora-B to regulate cell division. Similar to survivin, high expression of Aurora-B and INCENP are observed in cancer. In OSCC, clinicopathological significance of nuclear survivin expression is controversial and there is no report on the correlation between the expression of survivin and Aurora-B. In the present study, therefore, we examined the immunohistochemical expression of survivin and Aurora-B in cytoplasm or nucleus of OSCC and explored the relationship between their expression and clinicopathological factors for evaluating their predictive value. Our results confirmed that survivin expression was observed in both nuclei and cytoplasm of normal oral squamous epithelia and OSCC cases, but the number of positive cells was significantly higher in OSCC than in normal epithelium. OSCC also showed high expression of Aurora-B only in their nuclei and positive relationship between survivin and Aurora-B expression. The cases with high nuclear or cytoplasmic survivin expression and high Aurora-B expression showed poorly differentiated histology and high frequency of lymph node metastasis. Moreover, we found that Aurora-B expression was well correlated with cell proliferation and number of multinuclear cancer cells. In conclusion, our findings show that both cytoplasmic and nuclear survivin was related to lymph node metastasis in OSCC and nuclear survivin expression was associated with Aurora-B expression. We suggest that survivin and Aurora-B expression can be predictive markers for OSCC, and that association between survivin and Aurora-B may be involved in the progression of OSCC.

11-11

Dynamic Shear Properties of Porcine Mandibular Condylar Cartilage

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INTRODUCTION : Shear stress can result in fatigue, damage and deformation in the mandibular condylar cartilage. Therefore, the data on the shear behavior might to a better understanding of secondary tissue damage in the articular cartilage. In order to characterize these properties, we tested the shear response of the mandibular condylar cartilage from pigs over a wide range of loading frequencies.

METHODS : Ten porcine mandibular condyles were used for dynamic shear tests; two cartilage-on-bone plugs were dissected from each condyle. The specimens were clamped between the plates of a loading apparatus under a compressive strain of 10%. Dynamic shear was applied to the specimen by a sinusoidal strain of respectively 1.0%, 2.0%, and 3.0% in teh antero-posterior direction. Frequencies reanged between 0.01 and 10 Hz.

RESULTS : The magnitudes of the dynamic shear moduli $|G^*|$, G' and G'' were found to be dependent on the frequency and the amplitude of shear loading. The dynamic shear moduli significantly increased with shear strain. Meanwhile, tand ranged from 0.2 to 0.4, which means that the cartilage is primarily elastic in nature and has a small but not negligible viscosity.

DISCUSSION AND CONCLSIONS : The present results show that the dynamic shear moduli became larger with an increase of the frequency and amplitude of the applied strain, which is in agreement with the hyperelastic nature of cartilage. In future, to evaluate the anisotropic characteristic of the mandibular condylar cartilage, the dynamic shear properties of the cartilage in the mediolateral direction should be investigated. This may give more insight into the possible mechanisms of cartilage degradation due to shear recognized in the TMJ osteoarthritis

11-12

Distinct Roles of Synovial Membrane-Derived and Bone Marrow-Derived Mesenchymal Stem Cells in the Synthesis/Degradation of Hyaluronan-Containing Matrix

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Synovial fibroblasts (SF) proliferate in response to chronic inflammation, release matrix-degrading enzymes, such as matrix metaloproteases (MMPs), and form pannus in arthritic joints. We found that SF isolated from normal, OA (osteoarthritis) and RA (rheumatoid arthritis) synovium, like bone marrow meschenchymal stem cells (BM-MSC), can differentiate into osteoblasts, chondrocytes and adipocytes under appropriate culture conditions. Thinking of the multi-differentiation potential, these SF are MSC-like cells.

On the other hand, bone marrow aspirates and culture media conditioned by BM-MSC are viscous, perhaps because of the synthesis and accumulation of large hyaluronan (HA). Joint fluids also contain large HA at high concentrations (3-5 mg/ml), possibly due to enhanced synthesis of large HA and suppressed HA degradation. However, the HA size decreases in osteoarthritic (OA) and rheumatoid arthritic (RA) joints, and this decrease is thought to have a deleterious effect on joint elasticity and movement. Since the mechanism responsible for the accumulation of large HA in these fluids is unknown, we compared SF and BM-MSC to characterize their roles in turnover of HA and other matrix macromolecules.

We determined mRNA levels of HA-related genes in BM-MSC, normal SF, OA-SF, RA-SF and skin-fibroblasts. BM-MSC showed higher expression levels of HAS1 and HAS2, which are involved in the synthesis of large HA, than did other cells. In contrast, normal SF, OA-SF, and RA-SF showed higher expression levels of HYAL-1, CD44, HABP4, and TSG-6, which are involved in HA degradation, than did other cells. In addition, normal-, OA--, and RA-SF exhibited much higher expression levels of MMP1 and MMP3 in the presence or absence of IL-1. These findings proved that SF are unique MSC-like cells involved in the turnover of HA and other matrix macromolecules, although BM-MSC play a role in the accumulation of HA.

11-13

Expression and Roles of Toll-Like Receptors in Periodontal Tissue Cells

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keyword : toll-like receptor, innate immunity, LPS, CpG-ODN

BACKGROUND AND STUDY OBJECT : Innate immunity induced by periodontal pathogen is responsible for not only exclusion of bacteria but also establishment of consequent acquired immunity and plays important roles in host defense mechanisms and inflammatory reactions in periodontal tissue. Toll-like receptors (TLRs) are pattern recognition receptors, which provoke innate immunity. Especially, TLR2 (lipoteichoic acid), TLR4 (lipopolysaccharide; LPS), TLR5 (fragellin) and TLR9 (CpG-DNA) are important in immunoreactions of periodontal disease. The aim of this study was to examine the effects of TLR4 and TLR9 signaling pathway stimulation on periodontal tissue constitutive cells.

MATERIAL AND METHODS : TLRs mRNA expression in primary osteoblast (OB), OCCM-30 (a mouse cementoblast cell line; kindly provided from Prof. Somerman), MC3T3-E1, ST2 and Raw cells (mouse macrophage) was examined by RT-PCR. Cytokines (IL-6, RANKL and OPG), TLR4 and TLR9 mRNA expression levels in ST2 and OCCM-30 stimulated with *Actinobacillus actinomycetemcomitans*-LPS (100ng/mL, kindly provided from Prof. Nishihara) or CpG-ODN (ODN1826) stimulation were also assessed with real-time PCR.

RESULTS AND DISCUSSION :

1) All cells examined expressed TLR2, 4, 5 and 9 mRNA at various levels.
2) LPS caused up-regulation of IL-6 mRNA in ST2 and OCCM-30 (2hrs), while CPG-ODN did not induce any changes. In ST2, LPS-induced up-regulation of RANKL (24hrs) and down-regulation of OPG (2 and 24hrs) were evident, whereas RANKL and OPG were up-regulated in LPS-stimulated OCCM-30 (2hrs). CpG-ODN induced only down-regulation of OPG mRNA level in OCCM-30 (2 and 24 hrs). It is considered that activation of TLR4 or TLR9 pathways may be involved in periodontal tissue destruction through modulating cytokine production and RANKL/OPG balance.
3) LPS-stimulation significantly suppressed TLR4 and TLR9 mRNA levels in ST2 and OCCM-30. It is suggested that TLR4 pathway may negatively control excessive immunoreactions to periodontal pathogen via TLRs expression levels.

CONCLUSIONS : TLR signaling pathways may complexly regulate immunoreactions against attack of periodontal pathogen.

11-14

Effects of Lipid Compounds on Protein Kinase Activities and Ex Vivo Expansion of Mesenchymal Stem Cells in Serum-Free Medium

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Human bone marrow mesenchymal cells (MSC) are useful for treatment of bone and cartilage defects, periodontal diseases, and myocardiac infarction. Furthermore, MSC suppressed immunological rejection to allogeneic cells, and allogeneic transplantation of MSC supported hematopoiesis after transplantation of hematopoietic cells. The clinical application of MSC on regeneration medicine may extend to other diseases in future. However, MSC are a minor population of bone marrow cells and must be expanded ex vivo. Usually, MSC are expanded with 10~15% fetal bovine serum (FBS), but FBS could be contaminated by bovine spongiform encephalopathy prion and other unknown pathogens, and the composition of FBS is highly variable. Auto-human serum may be safe, but a large volume of blood has to be collected with pain, and the growth-stimulation activity of human serum varies with age and among individuals. Therefore, in order to reproduce MSC reliably and safely, a chemically defined serum-free medium is desirable. In this study, we developed a serum-free medium (STK2) that allowed better expansion of MSC than those grown in the presence of 10% FBS throughout successive passages, and these cells also maintained their multidifferentiation potential (osteogenic, chondrogenic and adipogenic) at high levels. Besides of the growth factors, several lipids were added in STK2 medium, and we investigated the effects of various lipid compounds in STK2 medium on continuous subculture and expansion of MSC. The results demonstrated that several lipid compounds were necessary for cell survival and proliferation. Furthermore, Western blotting indicated that phosphorylation of ERK1/2 and Akt was activated strongly by lipid compounds. The serum-free cultures should be useful for basic studies on MSC and allow us to expand transplantable MSC safely at a consistent growth rate.

11-15

Automutanolysin (Aml) Exhibits Lytic Activity Towards Planktonic and Biofilm Cells of Clinical Oral Streptococci

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Automutanolysin (Aml) of *Streptococcus mutans* was previously demonstrated to selectively lyse human cariogenic bacteria. Because of the explicit substrate specificity, Aml may represent an alternative way for the caries prevention. In this study, we investigated lytic activity of Aml against clinically isolated oral streptococci in planktonic and in biofilm conditions. We isolated oral streptococci from 53 patients (39 S. *mutans* and 9 S. *sobrinus* strains) of Hiroshima University Hospital. For lytic assay, the cells were suspended in lysis buffer (0.1M sodium phosphate buffer (pH 6.8) containing 100 mM NaCl and 0.1 mM $CaCl_2$ to an OD_{660} of 0.5). The suspension was mixed with Aml (final conc. 10 μ g/ml); incubated at 37℃ and the change of turbidity was monitored. In some experiment, lytic buffer containing non-ionic detergent (Triton X-100, final 0.1%) was used. For biofilm assay, bacteria were grown in Brain Heart Infusion broth containing 0.1% (wt/vol) sucrose in a 96-well plate. After biofilm formation, the wells were treated with the same buffers used in the lytic assay. To investigate lytic activity, we detected DNA by PCR that was released by Aml-induced lysis into buffer. In some experiments, reaction mixture was changed every 30 min and the change of biofilm density was monitored. Our results indicated that Aml has strong lytic activity, which was significantly potentiated in the presence of Triton X-100, towards the tested clinical strains in the planktonic condition. Moreover, Aml showed an ability to lyse bacterial cells in biofilm.

11-16

Chemotherapy and Radiation Against Neuroblastoma Inhibit Forming of Permanent Teeth

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PURPOSE : The aim of the present study was to assess the prevalence of dental abnormalities in children received chemotherapy and total body irradiation (TBI) against neuroblastoma.

METHODS : Five girls were participated in this study after receiving sufficient informed consent. They had been diagnosed as neuroblastoma in Stage IV, and were treated with chemotherapy and TBI in Department of Pediatrics, Hiroshima University Hospital. The mean age of their first treatment was 3.5 years old (range from 2.1 to 5.4). The abnormalities of teeth, such as anodontia, microdontia and tapered root, were surveyed morphologically by orthopantomographs.

RESULTS : Nineteen anodontia were recognized in 3 patients. Thirteen microdontia were found in 4 patients. Seventy-four teeth had tapered root among 78 permanent teeth seen on orthopantomographs from 5 patients. One patient presented only tapered root. Another one patient presented tapered root and microdontia. Three patients presented all abnormalities. Those abnormalities had appeared on orthopantomographs after 2 years received chemotherapy and TBI.

CONCLUSION : Tapered root was the most frequent abnormality found in children who underwent neuroblastoma treatment, and its prevalence was significantly high compare to healthy Japanese population. The progress of anti-neoplastic therapy causes new problem in our field. The abnormalities of permanent teeth must induce deterioration of quality of life in young patients. We should care them periodically for a long time.

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Transcription Factor, Runx3 Can Be a Useful Marker for Predicting Malignant Behavior of Oral Cancer

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Oral cancer is globally increasing and over a half million patients with oral cancer occur in a year. Early detection and treatment are important for QOL of oral cancer patients. Here we focused on transcription factor, Runx3, which has a Runt domain. Runx3 is known as a tumor suppressor gene in gastric cancer. In these tumors, reduced expression of Runx3 is frequently caused by methylation of its promoter lesion. However, it recently has reported Runx3 overexpression was observed in basal cell carcinoma of skin. We think that this discrepancy may be due to two different tissue types, glandular and squamous epithelium. Here we examined the expression and roles of Runx3 in oral cancer. First, we examined the expression of Runx3 in oral cancer by RT-PCR, Western blot and immunohistochemistry. We found that Runx3 was frequently overexpressed in oral cancer cells and tissues and was well correlated with differentiation and metastasis. Then, to know the roles of Runx3 in oral cancer, we generated Runx3-overexpressing cells. Runx3 overexpression enhanced cell proliferation. We confirmed this phenotype by using Runx3-siRNA. To know the mechanism of Runx3 for cell proliferation, we examined the gene expression profiles between control and Runx3 overexpressing cells by microarray analysis. In particular, we focused on Cyclin E among up-regulated genes, because Cyclin E is known as a positive regulator of cell cycle progression and frequently overexpressed in cancer cells.Interestingly, Runx3 overexpressing cells enhanced cyclin E expression, and Runx3-knockdown cells reduced cyclin E expression in comparison with control cells. Overall, Runx3 overexpression is involved in oral cancer development through enhancement of cell proliferation. We suggest that Runx3 can be a useful marker for predicting malignant behavior of oral cancer.

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