Transactions of The Showa University Society : The 317th Meeting

Special Lecuture

Mathematical Models for Population and Infectious Diseases

Hisashi Inaba
Graduate School of Mathematical Sciences,
University of Tokyo

In 1990s, the Japanese society received two big challenges in the fields of demography and epidemiology ; one is the second demographic transition (lasting below replacement fertility and rapid aging) and the second is the HIV/AIDS epidemic. I would like to report my mathematical models developed to quantify those population phenomena at that time and discuss the role of mathematical models to understand social, or biological phenomena.

First, I have developed an age-structured population model that recognizes the marital status of individuals, because the Japanese population has been reproduced mainly by legitimate first marriage. By the first marriage model, I have shown that the decline of the total fertility rate of Japanese women since 1975 can be explained by the delay of marriage and the increase of lifelong unmarried, for which the model is still applicable as a basic model for the future population projection.

Second, I have constructed an age-structured epidemic model to describe the invasion phase of HIV, where "age" indicates the infection-age (the time elapsed from the infection). In the initial invasion phase, the infected population can be described by the stable population model. Then I established a relation between the size of observed AIDS cases and the (unobservable) size of infected population in the incubation period. Using observed data of the Malthusian parameter and the survival rate, I gave an estimate for a possible size of HIV infections at that time.

After a quarter of century passed, these problems are not yet resolved and they are becoming more serious. The total population size of Japan has begun to decrease, and the number of HIV infections has been increasing. Although, in order to quantify those population problems and to consider the control policy, it was shown that mathematical population models are the most powerful, essential tool ; we still have no graduate program to educate demography, and mathematical epidemiology, is a very serious lack in Japanese universities.

Key words : population, demography, epidemiology, HIV, mathematical model

Research Introduction Lecture

To Create Oral Health Care through Oral Function

Shouji Hironaka
Department of Special Needs Dentistry,
Division of Hygiene and Oral Health,
Showa University School of Dentistry

Our division has been conducting education, research, and clinics on the theme of "Eat Orally throughout Life." This theme represents the general subject of three research studies. These studies include the 1) epidemiology and development of eating and swallowing function in children with disabilities, 2) the restoration of function and oral environments in the perioperative period, and 3) the identification of the mechanisms underlying the decline of eating and swallowing functions in frail or elderly people with dementia.

Our division also conducted research on the use of ultrasound for functional assessments. I wish to conduct research on Non-Invasive Functional Diagnosis Systems, including 4-D ultrasonography. In the future, I would like to develop screening methods, using Handy Ultrasonography, for use in public health settings. Next future vision of public health is the cooperation of the medical and industrial. We have already been

collaborating with the Department of Electronics and Engineering at Keio University. We developed a fiber grating swallowing sensor prototype. I think medical and industrial science cooperations could lead to robots for use in medical operations.

In the near future, our country is expected in shift medical emphases from medical care to nursing care Caring Care and from hospitals and medical facilities to home care. Medical professionals are responsible for caring for people in all phases of development and across generations. Therefore, we need to establish connections in the community and combine hospitals and clinic services as soon as possible. I would like to contribute to oral health care education and research.

Key words : eating, swallowing, oral function, oral health care, epidemiology

Original Sessions

1. Observation of Tooth-Coating Materials on Dentin by Experimental Swept-Source Optical Coherence Tomography

Eiko Mizukami[1], Yuka Gokan[1],
Atsufumi Manabe[1], Mayumi Chida[2],
Hideo Kakuma[2], Emi Hashimoto[3],
Kazuyuki Araki[3], Tsukasa Sano[3],
Takashi Miyazaki[4]

[1] Department of Conservative Dentistry,
Division of Aesthetic Dentistry and Clinical Cariology,
Showa University School of Dentistry
[2] The Yoshida Dental Mfg. Co., Ltd.
[3] Department of Oral Diagnostic Sciences,
Division of Radiology,
Showa University School of Dentistry
[4] Department of Conservative Dentistry,
Division of Biomaterials and Engineering,
Showa University School of Dentistry

Optical coherence tomography (OCT) is a non-invasive method that uses near-infrared light to produce tomographic images of tissue. OCT is widely used in clinical settings in ophthalmology. The aim of this study was to evaluate the effectiveness of an experimental swept-source optical coherence tomography

unit (SS-OCT, Yoshida Dental Mfg. Co., Ltd., Tokyo, Japan) for the observation of tooth-coating materials. Here, we observed dentin coated with a tooth-coating material. A tooth-coating material is commonly used in order to treat dentin hypersensitivity, mask the color of a darkened or stained tooth, and protect enamel and dentin. Nine materials were investigated : 3 could infiltrate dentin [Nanoseal (Nishika), MS Coat One (Sun Medical), Teethmate Desensitizer (Kuraray Noritake Dental)] and 6 were bonding/resin materials [Shield Force Plus (Tokuyama Dental), G-coat (GC), PRG Barrier Coat (Shofu), BisCover LV (Bisco), Beauti Coat (Shofu), White Coat (Kuraray Noritake Dental)]. We prepared 10 specimens by trimming and polishing freshly extracted human molars. No changes were observed in SS-OCT images of specimens coated with the 3 dentin-infiltrating materials. In contrast, reflection intensity was diminished in SS-OCT images of the specimens coated with the 6 bonding/resin materials. The materials that infiltrated dentin formed films approximately 1 μm thick, and the bonding/resin materials formed films greater than 10 μm thick. Therefore, these results indicate that the experimental SS-OCT unit could detect films greater than 10 μm thick.

Key words : SS-OCT, Tooth-coating Materials, Dentin

2. Examination of Optimal Sites and Loading Methods in Measuring Maxillary Complete Denture Retention

Takuya Kakuda, Yuji Sato,
Noboru Kitagawa, Momoe Nakatsu,
Kana Aoyagi, Takamasa Ogawa,
Mari Takayama, Kensuke Tsubakida
Department of Geriatric Dentistry,
Showa University School of Dentistry

Purpose

Retention and stability are essential for successful complete denture treatment. To evaluate denture retention, we developed a compact device to measure the retention factors of a denture and aimed to identify the optimal sites and loading methods for retention measurement.

Methods

This study included 30 subjects with edentulous

maxillae. Jigs coating dentures were made from a thermoplastic resin to protect and pull the denture. The pulling test was performed at the central point of the posterior border（P）, the intersection point of the line joining the right and left first molar central fovea（C）, and left first molar central fovea（MF）. The pushing test was performed at the central point of both the central incisor edge（IE）, and right first premolar buccal cusp（PC）. Each site was measured five times. Measurement was discontinued at 30 N due to pain. A site was considered immeasurable if it could not be measured twice in succession.

Results

The C and MF sites were unmeasured in about 50% of the subjects. The P, IE and PC sites were measured in all subjects. P and IE showed no significant difference. However, PC was significantly higher than P and IE. A positive correlation was observed between P and IE, and P and PC.

Conclusion

IE showed a strong positive correlation with P which has been suitable for reproducible measurements of denture retention forces. Therefore, the optimal site and loading method was considered to be the pushing test of IE without the jig.

Key words : Complete denture, Retention forces, optimal site

3. Clinical Dental Education by using iPad ―Virtual Tracing and Image Interpretation―

Yukiko Matsuda, Kenji Seki,
Yukinori Kimura, Kazuyuki Araki,
Tsukasa Sano
Department of Oral Diagnostic Sciences,
Division of Radiology,
Showa University School of Dentistry

From June 2013, PACS was introduced to our hospital. Diagnostic imaging has changed from a film to digital system. On clinical training in undergraduate education, in accordance with the changes to the digital system, a new construction of the system was required. We covered some of the educational program by construction of the iPad.

Virtual tracing on iPad

Students tried to trace the panoramic and intraoral image by installing the SketchBookX on the iPad. This SketchBookX is able to add the layer which was used as tracing paper on the image. Students traced the anatomical structures on a layer sheet. After the tracing was finished, the student saved the first layer image without a radiographic image and added a second layer sheet on the first layer sheet to add the anatomical structure names. These layer images were saved without a radiographic image and printed out for the students.

Interpretation trial

Trial A : Observing digital images on iPad. Students could adjust contrast, brightness and magnification of the image by using the iPhoto application. Interpretation report includes a handmade sketch image.

Trial B : Observing and tracing digital images on iPad. Interpretation report was made by hand.

Students remarked that Trial A was the easiest and it provided a good chance to learn about image adjustment. Some student's traced products with disease case were much better than expected in Trial B.

For students, this trial might offer a good chance to learn about change contrast, brightness and magnification for interpretation in digital radiographs.

Key words : digital radiographic image, virtual tracing, student

4. Diagnosis of root fracture using CBCT : Case presentation

Hitoshi Sakaue[1], Yoshio Yahata[1],
Masayuki Takabayashi[1], Yoshishige Yamada[1],
Yoshiko Masuda[1], Takashi Miyazaki[2]
[1] Department of Conservative Dentistry,
Division of Endodontology,
Showa University School of Dentistry
[2] Department of Conservative Dentistry,
Division of Biomaterials and Engineering,
Showa University School of Dentistry

Introduction

It's important to use intraoral radiography in endodontics to make a diagnosis. The location and extent of radiolucency in the intraoral radiograph indicate periapical periodontitis, perforation or root fracture. However, a diagnosis of root fracture is

difficult by intraoral radiograph. Cone beam computed tomography（CBCT）is very useful in the case of root fracture. In the present cases intraoral radiographs could not confirm the root fracture, but CBCT could confirm the root fracture.

Case 1 : the mandibular second molar had a vertical root fracture. CBCT detected the fracture line, while the intraoral radiograph did not show the fracture line.

Case 2 : the maxillary central incisor had a horizontal root fracture. CBCT detected the fracture line, while the intraoral radiograph did not show the fracture line.

Discussion

It is very important for the diagnosis of root fracture to be made as soon as possible. A tooth with a root fracture could make a large bone defect, and prosthodontic procedures may be difficult in the case of a large bone defect after extraction of the tooth with the root fracture. The definitive diagnosis of root fracture is the viewing of the fracture line. It is difficult to view the fracture line in the endodontic procedure in spite of using a dental operative microscope. In such cases, CBCT is helpful to detect the fracture line.

Conclusion

CBCT is useful in the endodontic preoperative examination and seems valuable to detect the fracture line that cannot be detected by using intraoral radiography.

Key words : root fracture, CBCT, intraoral radiography

5. A case report : Non-odontogenic Toothache Associated with Myofascial Pain of the Masseter Muscle
Yuki Watanabe, Masahiko Funato
Department of Special Needs Dentistry,
Division of Temporomandibular Disorders
and Orofacial Pain,
Showa University School of Dentistry

A 16-years-old man presented with mild to severe, variable but continuous, dull, aching pain diffusely located the left upper molar tooth, jaw, temple and difficulty of jaw opening.

The symptoms began 5days earlier ; his headache developed initially on the left temple and the left upper first molar toothache, and painful jaw and the difficulty of the jaw opening occurred the following at the family dentist. The patient underwent an examination and the teeth were fine. He was referred to Showa University Dental Hospital for the diagnosis of TMD.

Examination

Intraoral : There was no clinical or radiographic evidence for the pain. The maxillary left first molar and all left mandibular and maxillary teeth were not restoratively and endodontically treated, and responded normally to the percussion test and thermal test.

Masticatory muscle : Severe palpable tenderness in the left temporalis and masseter muscles.

From the left masseter trigger point, there was referred pain in the maxillary left molar teeth and the temporal region ; and the pain felt similar to a toothache and headache.

Treatment and Prognosis

It was explained that the toothache and headache are referred from the myofascial pain of the masseter muscle and the patient was instructsd self-care for the muscle disorders.

At X＋21-day re-examination, the jaw range of motion without pain was 55 mm, and myalgia of the masseter muscle, the toothache and headache were improved.

Conclusion

Various pain symptoms orally and on the face may be derived from myofascial pain of the masticatory muscle.

Key words : non-odontogenic toothache, TMD, myofascial pain, referred pain, headache

6. Features of Dental Hospital for Psychiatric Patients ─A case of Showa University Karasuyama Hospital─

Mitsuhiro Matsuura[1], Mie Myers[1],
Yasushi Nagao[1], Yuriko Ando[1],
Marina Kobayashi[1], Ayumi Oki[1],
Shohei Matsui[1], Hanon Katayama[1],
Yasubumi Maruoka[1], Asako Yamaguchi[2],
Kunie Hiyama[2], Shinji Nozue[3],
Madoka Yamashita[3], Koji Takahashi[3]

[1] Department of Special Needs Dentistry,
Division of Community-Based Comprehensive Dentistry,
Showa University School of Dentistry
[2] Dentistry and Dental Surgery,
Showa University Karasuyama Hospital
[3] Department of Special Needs Dentistry,
Division of Rehabilitation Medicine,
Showa University School of Dentistry

It is said that are about 3,200,000 psychiatric patients in Japan. However, there are reports of 8,000,000 people more given the inclusion of the boundary region in dementia patients. Psychiatric patients will increase in the future.

Showa University Karasuyama Hospital opened in 1951. It is a psychiatric hospital. Also the clinical research institute of clinical pharmacology and therapeutics clinical research center, the internal medicine, and dentistry and dental surgery have been established. Numerous psychosis patients, with disorders such as, schizophrenia, dementia, bipolar affective disorder, alcoholic psychosis, and Asperger's syndrome are treated there.

Dentistry and psychiatry are deeply related. Many psychiatric patients have very poor oral hygiene. Thus dental treatment and follow-up of oral health care is very important.

In addition, various accidents or incidents occur in a psychiatric hospital ; suffocation is one such incident. It is a severe problem for hospitals. Serious accidents or incidents due to suffocation occurred there in fiscal 2012. In cooperation with the medical staff of other departments, we made efforts to prevent suffocation. As a result, serious incidents or accidents due to suffocation did not occur in fiscal 2013.

Also patients with dental phobia have increased in recent years. We are doing dental treatment with intravenous sedation for such dental phobia patients.

Key wards : psychiatric patient, dementia, dental care

7. Clinico-statistical survey of patients in three years at Showa University Northern Yokohama Hospital dentistry and oral surgery after opening

Sayaka Yoshiba[1,2], Ayako Miyakubo[1],
Ken Yuasa[1], Naoko Kimura[1],
Mei Shoji[1], Kazuha Watanabe[1],
Maiko Suzuki[2,3], Hitoshi Watanabe[1,2,3],
Tatsuo Shirota[2]

[1] Dentistry and Oral surgery,
Showa University Northern Yokohama Hospital
[2] Department of Oral and Maxillofacial Surgery,
Showa University School of Dentistry
[3] Dentistry and Oral surgery,
Showa University Koto Toyosu Hospital

Showa University Northern Yokohama Hospital was established in 2001 as a regional core hospital located in the Northern Yokohama area. We aimed to consider the future prospects of oral care and oral surgery in Showa University Northern Yokohama Hospital and therefore carried out a clinico-statistical survey of the patients in the three years after opening.

The results of the survey showed that the total number of new patients from 2011 to 2013 was 3164. Regional and hospital referred new patients were 1881 and 1283, respectively. The most frequent classification was dental- periodontal disease in both groups. Regional referral patients included oral surgery treatment, whereas hospital referral patients included operative and prosthetics dentistry. Hospitalized patients increased during this three-year period, and the total number of hospitalized patients was 150. The average number of days in hospital was 3.25. Compared to the number of days in hospital by disease classification, the number of days in hospital tended to increase in the order of oral mucosal disease, malformation-deformation disease, and inflammation.

A strong collaboration system of vicinity medical facilities has been developed, and the emphasis is placed on the role as a secondary medical institution. As

dental professionals we assist in contribution to the medical teams of other occupations.

8. Development of Chair Side Clinical Pathways in Complete Denture Adjustment

Mariko Ishikawa, Yuji Sato,
Noboru Kitagawa, Toshiharu Shichita,
Yukari Isshiki, Daisuke Kawata
Department of Geriatric Dentistry,
Showa University School of Dentistry

Purpose

Clinical pathways are structured, treatment plans designed to support the implementation of clinical guidelines and protocols. Clinical pathways for dentistry has been used primarily at the time of surgery and hospitalization for oral surgery, although to date there are few reports on the use of clinical pathways in general dentistry. An introduction to clinical pathways would improve the efficiency and standardization of dental treatment. The purpose of this study was to develop chair side clinical pathways in complete denture adjustment.

Methods

The subjects were 61 patients with complete dentures (31 patients before clinical path introduction, 30 patients and after clinical pathways introduction). Before clinical pathways introduction, investigations were performed at the time of complete denture adjustment by dentists and assistants. The assistants were also dentists. A clinical pathways was developed to make denture adjustment steps more efficient. In particular, dentists and assistants planned to share the dental treatment steps, and patients were to respond to a questionnaire in blank time. Investigations were repeated with the clinical pathways.

Results

No significant difference in chair time was found between before and after the introduction of clinical pathways (Levene test). Average blank time was significantly reduced 9.0 minutes in dentists, 10.2 minutes in patients, and 12.1 minutes in assistants (Mann-Whitney test). By developing and introducing the chair side clinical pathways, the efficiency and standardization

of dental treatment will be improved.

9. Efforts and establishment of maternity dental program in Showa University Northern Yokohama Hospital : The results of the questionnaire after participation in the class for mothers and babies

Mei Shoji[1,2], Naoko Kimura[1,2],
Ayako Miyakubo[1,3], Kazuha Watanabe[1,2],
Ken Yuasa[1,4], Sayaka Yoshiba[1,5],
Hitoshi Watanabe[1,5,6], Ayumi Oki[3],
Tomomi Sugiyama[7], Mari Fujioka[7],
Mitsuko Inoue[7]
[1] Dentistry and Oral Surgery,
Showa University Northern Yokohama Hospital
[2] Division of Dental Hygienist,
Showa University Dental Hospital
[3] Department of Special Needs Dentistry,
Division of Community Based Comprehensive Dentistry,
Showa University School of Dentistry
[4] Department of Special Needs Dentistry,
Division of Oral Rehabilitation Medicine,
Showa University School of Dentistry
[5] Department of Oral and Maxillofacial Surgery,
Showa University School of Dentistry
[6] Dentistry and Oral surgery,
Showa University Koto Toyosu Hospital
[7] Department of Pediatric Dentistry,
Showa University School of Dentistry

The oral health program for maternity dental support opened in Showa University/Northern Yokohama Hospital in September, 2012. The program was designed for women in 16–24 weeks of pregnancy and receiving prenatal care in our hospital.

In August 2013, we started "the class for your baby". This class targeted mothers and babies in the primary teeth eruption period, which is about 6 months after the baby's birth. This class provided guidance about the function and development of the infant oral formula, when and how to provide weaning food, importance of mouth play and the needs and procedure of oral care.

This study was carried out from the questionnaire

answered by 58 mothers attending the program "the class for your baby" during August 2013 and May 2014.

Mothers were mostly 35 to 39 years old and babies were mostly 6 months old.

For the question of "what is of concern or causes worry", most participants answered weaning foods and the completion of weaning. For the question of "What you are concerned about with your baby's mouth and teeth", most answers were dental caries, teeth alignment and tooth brushing. And also, most of the mothers answered that they were satisfied with the program.

From these results, it is reasonable to think that concerns and interests of the participants are consistent with what has been taught at "the class for your baby". Therefore, the maternity dental program has been able to meet the needs of pregnant women who participated in this program.

Key words : maternity dental program, pregnant woman, baby

10. The analyzed behavior of MC3T3-E1 cell cultured on the ceria stabilized zirconia / alumina nanocomposite

Yoko Oshima, Fuminori Iwasa,
Shinpei Tanaka, Keita Tachi,
Ikuyo Oono, Kazuyoshi Baba
Departments of Prosthodontics,
Showa University School of Dentistry

Objective

Ce-TZP/A which is composed of nano-sized grains and particles dispersed in each crystal and has therefore higher strength and fracture toughness than conventional Y-TZP, has been widely used for crowns and bridges. Since Ce-TZP/A has low susceptibility to hydrothermal degradation, this material has the potential to be applied to dental implant fixtures. This study aimed to investigate the initial response of MC3T3-E1 for hydrofluoric treated Ce-TZP/A with nanostructure surface.

Methods

Ce-TZP/A disks with two different surface modifications (4% hydrofluoric acid : 4%Zr, and 55%HF etching : 55%Zr) were prepared. MC3T3E-1 cells were cultured on these disks and on acid-etched titanium (AETi) as a control. The culture on each disk was examined by the WST-1 test for cell attachment and by alkaline phosphatase staining for cell differentiation. Cell spread and cytoskeletal development were evaluated by fluorescence imaging.

Results

SEM showed that the HF-etched Ce-TZP/A surface had globular structures containing irregularities. The incidence was greater with 55%Zr than with 4%Zr. Cell attachment and differentiation were consistently enhanced on the 55%Zr compared with AETi. By fluorescence imaging with actin filament and vinculin staining, cell processes and cytoskeletons developed more extensively on the HF-etched Ce-TZP/A surface than on the AETi, irrespective of HF concentration.

Conclusion

A newly created nanostructure surface on zirconia/alumina composites enhances initial attachment, cytoskeletal organization and eventually differentiation in osteoblasts.

Key words : zirconia implant, cell attachment, cell differentiation, osteoblast

11. Oral cancer detection by Activated Fluorescent Probe-γ-Glutamyltranspeptidase

Masahiro Nagasaki, Daisuke Soga,
Sayaka Yoshiba, Sunao Shiogama,
Seiji Kondo, Tatsuo Shirota
Department of Oral and Maxillofacial Surgery,
Showa University School of Dentistry

Currently, there are methods available for the detection of human cancer such as X-ray, computed tomography (CT), ultrasonography (US), and magnetic resonance imaging (MRI). However, these methods are not capable of precisely describing the boundaries between cancer and normal tissues. In oral cancer treatment, it is important that we completely remove the primary lesion and metastasis. Therefore, the visualization of the cancer cells is critical as the new cancer cell detected method to determine the most appropriate excision range in operations.

γ-glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) is acolorless and non-fluorescentee reagent, but it can specifically make cancer cells, whose γ-glutamyl

transpeptidase（GGT）activity is high, become fluorescent.

In this study, we confirmed the activation of GGT in 8 human oral squamous cell carcinoma（OSCC）cell lines（SAS, SAS L-1, HSC-2, -3, -4, NA, SCC-25, -111）*in vitro* and the mouse xenograft models（balb/c/nu/nu）*in vivo*. OSCC cell lines and mouse xenograft models could specifically confirm fluorescence by gGlu-HMRG probe.

Furthermore, we confirmed whether it emitted light for the fluorescence from 0 to 60 minutes using the OSCC tissues.

As a result, we could confirm that light gradually became strong until 60 minutes after the cancer tissues were added to the gGlu-HMRG probe.

In conclusion, the gGlu-HMRG probe might be a useful clinical diagnostic tool and it is expected to help oral surgeons.

Key words : oral cancer, γ-glutamyltranspeptidase（GGT）, γ-glutamyl hydroxymethyl rhodamine green（gGlu-HMRG）, fluorescence imaging

12. Analysis of osteoclast precursors in bone marrow, spleen and blood

Takuya Enomoto[1], Masamichi Takami[2], Ryutaro Kamijo[3], Matsuo Yamamoto[1]

[1] Department of Periodontology, Showa University School of Dentistry

[2] Department of Pharmacology, Showa University School of Dentistry

[3] Department of Biochemistry, Showa University School of Dentistry

Osteoclasts are bone-resorbing giant cells which are differentiated from a monocyte/macrophage lineage, and involved in inflammatory bone destruction accompanied by periodontitis. Recent studies have shown that osteoclast precursors reside not only in bone marrow, but also peripheral blood and the spleen, though their precise characteristics are unknown. To analyze their characteristics, we isolated the cells using a cell separation system with magnetic bead-labeled antibodies against cell surface molecules such as c-Fms（M-CSF receptor）, RANK（RANKL receptor）, CD14 and CD11b（monocyte/macrophage markers）. Then, we cultured the cells in the presence of osteoclast

differentiation factors such as RANKL and M-CSF to determine their potential for osteoclast differentiation. We found that the osteoclast precursors in bone marrow were c-Fms（＋）, RANK（－）, CD14（＋）and CD11b（－）. The precursors in the spleen were c-Fms（＋）, RANK（－）, CD14（－）and CD11b（－）. The precursors in blood were c-Fms（＋）, RANK（－）, CD14（＋）and CD11b（＋）. These results show that c-Fms is commonly expressed on osteoclast precursors in each tissue, whereas CD11b is only expressed on the precursors in the blood. Flow cytometric analysis showed that CD11b（＋）cells in bone marrow and spleen did not express c-Fms. However, when LPS was administrated to mice, CD11b（＋）osteoclast precursors expressing c-Fms alternatively appeared in bone marrow and spleen. These results suggest that characteristics of osteoclast precursors in bone marrow, spleen, and blood are not the same, and inflammatory stimulation by LPS induces an alternative type of osteoclast precursors in the bone marrow and spleen, which may contribute to inflammatory bone destruction.

Key words : osteoclast precursors, LPS, CD11b, c-Fms

13. Induction of osteoblast differentiation from neural crest-derived cells in the hair follicle

Eri Morisawa-Urano[1], Masamichi Takami[2], Tetsuo Suzawa[3], Ryutaro Kamijo[3], Kazuyoshi Baba[1]

[1] Department of Prosthodontics, Showa University School of Dentistry

[2] Department of Dental Pharmacology, Showa University School of Dentistry

[3] Department of Biochemistry, Showa University School of Dentistry

Various kinds of scaffold, autologous bone, and tissue stem cells have been used to regenerate bone tissues. However, rejection and surgical stress for the patient sometimes becomes a serious problem. To resolve this, we tried to induce osteoblasts from hair follicle cells to use them as a cell source for bone regeneration. Hair follicles originate from the neural crest（NC）that appears in early stage of embryogenesis. Neural crest cells（NCCs）migrate throughout the embryo, and differentiate into various kinds of cell types such

as neuron, bone, teeth, thymus, and hair. In adult tissues, some NC-derived cells exist as tissue stem cells. In the present study, we succeeded to induce osteoblast-like cells from NC-derived whisker follicles in mice. We collected and cultured NC-derived hair follicle cells labeled with EGFP in the P0-Cre/floxed-EGFP double transgenic mice. The EGFP-positive hair follicle cells（NC-derived cells）continuously expressed Runx2 mRNA. Stimulation of the cells with BMP-2 induced expressions of alkaline phosphatase, osteocalcin, and osterix mRNAs and produced mineralized matrices positively detected by alizarin red and von kossa staining. The EGFP-positive hair follicle cells also continuously produced M-CSF and OPG. The addition of 1,25（OH)$_2$D$_3$（10^{-8}M) to the cultures suppressed OPG expression and induced RANKL production in the cells. When bone marrow cells were co-cultured with EGFP-positive cells in the presence of 1,25 （OH)$_2$D$_3$, multinucleated osteoclasts appeared within 10 days. These results indicate that NC-derived hair follicle cells possess a capacity to differentiate into osteoblasts, which will be beneficial for the development of a method for bone regenerative medicine.

Key words : Neural crest, hair follicle, osteoblasts