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Global Network Initiative for BioDental Education and Research

Hiroshima University Faculty of Dentistry

5th Hiroshima Conference on Education and Science in Dentistry

Proceedings of 5th Hiroshima Conference on Education and Science in Dentistry
October 12-13, 2013, in Hiroshima, Japan

Hiroshima University Faculty of Dentistry

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PREFACE

On behalf of organizing committee members of Hiroshima Conference and Hiroshima University Faculty of Dentistry, it is my great pleasure of extending to you an invitation to participate in 5th Hiroshima Conference on Education and Science in Dentistry with the theme, "Global Network Initiative for BioDental Research and Education" to be held in Hiroshima, Japan on October 12 and 13, 2013.

After eight years since 2006, Hiroshima Conference has become a well-recognized international meeting giving participants valuable opportunities to learn the up-to-date cutting edge dental science and education, to exchange new ideas and information, and to build research or academic ties among participating institutions and their scholars. Together, we have scheduled a forum for young scientists and students who will be leaders of next generation. In addition, we have scheduled a satellite international symposium of food, nutrition and health on October 14, 2013. This is the first international symposium with interdisciplinary theme about food, nutrition and health organized by School of Oral Health, Hiroshima University Faculty of Dentistry in collaboration with Hiroshima Prefectural University and Hiroshima Jogakuin University.

Please enjoy special lectures, symposiums and workshops and I wish you will be a part of this conference and experience memorable days as a speaker, a poster presenter or an audience.

With best regards,

Professor Motoyuki Sugai

President of 5th Hiroshima Conference on education and science in dentistry

Dean, Faculty of Dentistry Hiroshima University

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Making Universities More Global

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President of Hiroshima University

1. ONGOING GLOBALIZATION IN HUMAN SOCIETY

Rapid progress in academic research is resulting in the globalization of human society at an accelerated pace. In a world that is becoming more and more borderless, where free cross-border mobility of people, goods and cultures is possible, humans are expected to understand one another, irrespective of national borders, religions and cultures. Against this backdrop, cross-cultural experience is becoming increasingly important for young people, who are expected to shape the future society. At the same time, as the world becomes more global, it is becoming increasingly important for Japanese people to rediscover their unique characteristics, which are referred to in *Bushido: The Soul of Japan by Inazo Nitobe* and *The Book of Tea* by Tenshin Okakura. Today's globalized world is troubled by frequent regional conflicts caused by differences in religion and values. While progress in academic research has brought many benefits to us, it has also created new problems, such as air pollution, environmental destruction, depletion of energy and food resources, and terrorism.

Ongoing globalization in human society is now posing many new challenges that we must overcome as we strive to make our society safer and more peaceful.

Looking at Japan, as data presented at a meeting of the Industrial Competitiveness Council shows, among all developed nations, Japan is the only one that ranks lower and lower each year in the innovation category of the global competitiveness rankings. Japan's global market share of car navigation systems, lithium-ion cells, DVD players, liquid crystal panels and other electronics products continues to decline. Under such circumstances, Japanese companies are expanding overseas, especially in Asia. Over the past ten years, the number of Japanese companies operating overseas increased—from 6,345 to 11,497 in Asia, from 738 to 972 in Latin America, from 2,147 to 2,536 in Europe, and from 2,596 to 2,860 in the U.S. Meanwhile, more than 70 percent of Japanese companies that have overseas business facilities recognize that they need to employ or develop Japanese people who will help make their organizations more global.

2. HIROSHIMA UNIVERSITY'S EFFORTS TO MAKE ITSELF MORE GLOBAL

Hiroshima University dates back to 1874 when its oldest predecessor, Hakushima School, was established. After many transitions, eight academic institutions merged to form Hiroshima University in Hiroshima City, the first atomic-bombed city in the world, in 1949. The founding spirit of the University is "a single unified university, free and pursuing peace," and the first of its five guiding principles is "the Pursuit of Peace." In accordance with the founding spirit and the first guiding principle, in 1975, Hiroshima University established the Institute for Peace Science, the first of its kind that has been established by a national university in Japan. Five years ago, the University began to require its newly-enrolled students to visit various facilities that remind them of the importance of peace, such as the Hiroshima Peace Memorial Museum, Yamato Museum and Okunoshima Poison Gas Museum, and to submit reports on such visits. Then, two years ago, the University began to require its newly-enrolled students to study a peace subject of their choice from the liberal arts. In this way, the University has introduced into its curricula the subjects and programs that help students learn more about peace, expecting them to continue thinking about world peace even after graduation.

Moreover, to cope with a changing human society, Hiroshima University is making various campus-wide efforts to promote international student exchanges. Three years ago, the University launched a two-week overseas study program called the START Program for newly-enrolled students with little or no overseas experience. They study at partnership universities in the U.S., Australia, Indonesia, Vietnam, Taiwan and various other countries and regions, and interact with local students and residents. I hope that the students who participate in this program will study abroad on a mid- to long-term basis in the future. Students can also participate in other overseas study and internship programs, including: language training and cultural study programs at universities in the U.S., U.K., Germany, France, South Korea and China; International Network of Universities (INU) Double Degree Program (credit transfer available); Hiroshima University Study Abroad (HUSA) Program; University Studies Abroad Consortium (USAC) student exchange program; and Global Internship Program (G.ecbo). Due to the variety of programs available, the number of Japanese students studying abroad has recently doubled.

Hiroshima University also focuses on accepting more overseas students. The University now has about 800 regular students from overseas. However, most of them are graduate students. To attract students who will study on a mid- to long-term basis, the University is improving the living and learning environment for international students, such as provision of comfortable rooms, financial support and a two-week Japanese language and culture study program. The University is aiming to triple the number of enrolled international students over the next five years by enabling more students to take entrance examinations at the Hiroshima University Beijing Research Center, and venues in

Vietnam, Indonesia and other countries. Although the University will strive to attract overseas students mainly from East and Southeast Asia, it must also consider accepting more students from Europe, where graduates are having difficulty finding jobs, as well as from Africa and Latin America, which are expected to grow in the future. Hiroshima University accepts international students with support from the Hiroshima prefectural government and other local organizations.

3. NEED FOR INTERNATIONAL EXCHANGES IN THE FUTURE

The ideal human society is a safe, peaceful society where the gap between rich and poor is narrow, everyone enjoys good health, and environmental pollution problems as well as food, water and energy shortage problems have been resolved. To create such a society, all humans must help resolve these problems. To that end, we must further promote international exchanges, creating an environment where all people can understand, accept and support one another. It is particularly important for young people, who will shape our future society, to understand and accept one another's differences through interaction, thereby living together in harmony. Therefore, international inter-university exchanges are becoming increasingly important.

Japan has publicized its nationwide project for doubling the annual number of its students studying overseas to 120,000 in 2020 from the present 60,000. To achieve the goal of this project, specific proposals have been made: helping students learn useful English, assisting universities in improving their educational programs, creating an environment where study-abroad experience can lead to employment, and providing financial support. At the same time, Japan is working on a project for increasing the annual number of international students in Japan to 300,000 in 2020. To achieve the goal of this project, Japan is making various efforts, including: establishing facilities in overseas priority regions to attract local students to Japan; conducting promotional activities through these overseas facilities; helping Japanese universities improve their educational environment (introduce flexible academic schedules, number subjects according to academic area, level, etc); providing more scholarships; and establishing networks of international students who have studied in Japan.

People with different cultural backgrounds can understand and accept one another through interaction, thereby being able to live together in harmony on this planet. Then they can work together to contribute to society and build world peace. This is why universities need to become more global.

Special Lecture

Special Lecture I

Resolution of Inflammation in Periodontitis: a Potential New Treatment Paradigm

The Forsyth Institute

T.E. Van Dyke

Special Lecture II

Liver Immunity and Surgery

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Canadian Institutes of Health Research, University of Toronto

J.E. Aubin

Resolution of Inflammation in Periodontitis: a Potential New Treatment Paradigm

T.E. Van Dyke

DDS, PhD, VP for Clinical and Translational Research, Chair, Department of Applied Oral Sciences, The Forsyth Institute, Cambridge, MA 02132, USA.

ABSTRACT

The pathogenesis of periodontitis involves a complex immune/inflammatory cascade that is initiated by the bacteria of the oral biofilm that forms naturally on the teeth. Susceptibility to periodontitis appears to be determined by the host response; the nature and intensity of the inflammatory response and the differential activation of immune pathways. The role of innate immunity, failure of acute inflammation to resolve becoming chronic, cytokine pathways that regulate the activation of acquired immunity and the cells and products of the immune system have been the focus of our laboratory for many years. New information relating to regulation of both inflammation and the immune response has revealed the context of susceptibility to, and perhaps control of, periodontitis. Our recent data suggests that susceptibility to periodontitis is determined to some extent by the failure of active pathways of resolution of inflammation. Low molecular weight lipid mediators derived from eicosanoid pathways are central to our understanding of inflammation regulation in periodontitis and the potential of host modulating agents in the treatment of periodontal diseases.

INTRODUCTION

Inflammation is a physiological response to a variety of injuries or insults, including heat, chemical agents, or microbial infection. The acute phase inflammatory response is rapid and of short duration. If the insult or injury is not resolved, the response becomes chronic, which is non-physiologic or pathologic. When inflammation becomes chronic, the adaptive immune response is activated; the cellular and non-cellular mechanisms of acquired immunity are involved. Immune mechanisms further play a role in the resolution of inflammation and healing process, which includes repair and regeneration of lost or damaged tissues. Thus, innate (inflammatory) and acquired immunity must be coordinated to return the injured tissue to homeostasis[1].

The etiology of periodontal diseases is bacteria. The human oral cavity harbors a substantial and continuously evolving number of microbial species. The ecological interactions between the host and microbes define disease. Unlike many infectious diseases, periodontal diseases appear to be infections mediated by the overgrowth of commensal organisms, rather than the acquisition of an exogenous pathogen. As microorganisms evolve more rapidly than their mammalian hosts, immune mechanisms that determine the ecological balance of commensal organisms need to change as well to preserve homeostasis[2].

Regulation of inflammatory responses is critical to the understanding of the pathogenesis of complex diseases such as periodontitis. The pathogenesis of periodontal diseases appears to result from the inflammatory response to bacteria in the dental biofilm. The identification of the specific pathogens has been elusive. There is evidence that specific microbes are *associated* with the progressive forms of the disease; however, presence of these microorganisms in individuals with no evidence of disease progression suggests that the disease is the net result of the immune response and the inflammatory processes, not the mere presence of the bacteria. Regulation of immune-inflammatory mechanisms governs patient susceptibility and is modified by environmental factors[3-5]. Until fairly recently, the reasons for lack of inflammation control in periodontitis were unknown. The focus of research was proinflammatory mediators and cytokines and their relationship to the bacteria found at sites of disease. The discovery of the proresolution pathways by Charles N. Serhan, PhD in the 1990s opened new doors to our understanding of how periodontitis pathogenesis works.

Lipid Mediators of Inflammation

Prostaglandins (PGs) are derived from hydrolysis of membrane phospholipids. Phospholipase A_2 cleaves the *sn*-2 position of membrane phospholipids to generate free arachidonic acid, a precursor of a group of small lipids known as eicosanoids[196]. Arachidonic acid is metabolized by two major enzyme pathways. Lipoxygenases catalyze the formation of hydroxyeicosatetraenoic acids (HETEs) leading to the formation of proinflammatory leukotrienes (LT). Cyclooxygenases (COX-1 and COX-2) catalyze the conversion of arachidonic acid into proinflammatory prostaglandins, prostacyclins and thromboxanes. Prostaglandins have 10 sub-classes, of which D, E, F, G, H and I are the most important[2,6]. Inflamed gingiva synthesizes significantly larger amounts of prostaglandins when incubated with arachidonic acid than does healthy gingiva[7]. Prostaglandin E_2 (PGE_2) is a potent stimulator of alveolar bone resorption[8,9]. Within gingival lesions, prostaglandin E_2 (PGE_2) is mainly localized to macrophage-like cells and secreted when stimulated with bacterial Lipopolysaccharide (LPS)[200]. Periodontal ligament cells also produce prostaglandin E_2 (PGE_2) even when unstimulated. This secretion is enhanced by IL-1β, TNF-α and parathyroid hormone[11-13].

It is important to note that prostaglandin E_2 (PGE_2)

has biphasic actions in immune function. In high doses, it decreases immunoglobulin G (IgG) levels, but at low doses it has the potential to increase IgG. When combined with IL-4, low doses of PGE_2 induce a synergistic rise in IgG production suggesting an immune-regulatory role for prostaglandin E_2 (PGE_2)[14].

Periodontal inflammation begins as a protective response to bacterial biofilm. In susceptible individuals, periodontal inflammation fails to resolve and chronic inflammation becomes periodontal pathology. Periodontal disease results from excess inflammation and may be considered a failure of resolution pathways. An essential goal of interventions in inflammatory disease is the return of tissue to homeostasis, defined as an absence of inflammation. Hence, the rapid and complete elimination of invading leukocytes from a lesion is the ideal outcome following an inflammatory event[253]. Accordingly, inadequate resolution and failure to return tissue to homeostasis results in neutrophil-mediated pathology and chronic inflammation[16], with destruction of both extracellular matrix, and bone, scarring and fibrosis[17]. Scarring and fibrosis in periodontitis prevent the return to homeostasis[15].

The efforts to control inflammation to date have been focused on the use of pharmacologic agents that inhibit pro-inflammatory mediator pathways, e.g., non-steroidal anti-inflammatory drugs (NSAIDs)[18]. NSAIDs target COX-1 and COX-2–dependent pathways inhibiting generation of prostanoids. Newer classes of inhibitors target lipoxygenase pathways and leukotriene (LT) production or the actions of TNFα with receptor antagonists. The side effect profile of these agents prohibits their extended use in periodontal therapy.

More recent discoveries have uncovered the natural pathways of resolution of inflammation, which are an extension of the same eicosanoid pathways that produce proinflammatory mediators. The physiologic end of the acute inflammatory phase occurs when there is a "class switch" of eicosanoid pathways in neutrophils[15,19]. This class switch is mediated by the up-regulation of 15-lipoxygenase (15-LO) by neutrophils late in inflammation. Neutrophils in the early acute phase produce only 5-LO for the production of leukotrienes. The 15-LO catalyzes a second reaction with hydroxyeicosatetraenoic acid (HETE) products generated earlier by the neutrophil or other cells[20]. The series of enzymatic reactions starts with the oxidation of arachidonic acid (AA) by a lipoxygenase (5-, 12, or 15-LO, depending on the cell of origin). A 5-, 12-, or 15-S-hyroxy-(p)-eicosatetraenoic acid (15-S-H(p)ETE) intermediate is produced, which is then further acted on by a second lipoxygenase to induce the synthesis of doubly substituted intermediates (5, 15 H(p)ETEs for example) that are further metabolized into lipoxins, such as lipoxins A_4 (LXA_4) and B_4 (LXB_4)[16,21]. Lipoxins are receptor agonists that stimulate the resolution of inflammation and promote the restoration of tissue homeostasis through a number of mechanisms. These include limiting polymorphonuclear neutrophil (PMN) migration into sites of inflammation, modulating the phenotype of macrophages and stimulating the uptake of apoptotic neutrophils (PMN) without secretion of proinflammatory cytokines[22-24].

Unlike other non-steroidal anti-inflammatory drugs, aspirin has unique characteristics. Aspirin (ASA) acetylates the COX-2 enzyme to inhibit further production of prostanoids from AA metabolism, but the acetylated COX-2 acquires new enzyme activity as a 15-epi-LO. This alternative pathway leads to the synthesis of 15-*R*-H(p)ETE. This molecule transforms to 5(6)-epoxytetraene with the help of 5-LO. The product is 15-epi-lipoxins, also known as aspirin triggered lipoxins (ATLs)[16]. ATL, the 15R-epimer of native lipoxin, possesses more powerful pro-resolving properties due to increased half-life[16,25,26].

Lipoxins are the natural proresolving molecules derived from endogenous fatty acids, primarily arachidonate. Dietary fatty acids of the omega-3 class are also metabolized by similar pathways and the products (resolvins and their aspirin triggered derivatives) have similar biologic activity to lipoxins[15,27]. Resolvins stimulate the resolution of inflammation through multiple mechanisms, including preventing neutrophil penetration, phagocytosis of apoptotic neutrophils to clear the lesion, and enhancing clearance of inflammation within the lesion to promote tissue regeneration[28-30]. Interestingly, the classic inflammatory eicosanoids (*i.e.*, prostaglandins and leukotrienes), in addition to activating and amplifying the cardinal signs of inflammation, are responsible for inducing the production of mediators that have both anti-inflammatory and pro-resolution activities reinforcing the active nature of the resolution process[31]. In an animal model of periodontitis, treatment with resolvin-E1 completely eliminated signs of inflammation enabling regeneration of lost tissues[28] (Figure 1).

The demonstration that exogenously added lipoxins and resolvins can reverse periodontitis in animal models has opened a new field in regenerative medicine; the use of agonists of resolution of inflammation to control unwanted inflammation without inhibition of the desirable aspects of the acute inflammatory response; hence, the distinction between inhibition of inflammation and resolution of inflammation. Resolution of inflammation is an active, receptor driven program that occurs *after* the acute phase driving the lesion to homeostasis. This includes the return of the oral biofilm to a composition compatible with health. These data strongly suggest that much of the dysbiosis of the oral biofilm observed in periodontitis is actually the result of uncontrolled inflammation. In other word, in the absence of inflammation, the pathogenic biofilm cannot persist.

CONCLUSION

New data from our group have put into question several commonly held paradigms for the pathogenesis of periodontitis and therefore the treatment of periodontitis. In the near future, agonists of resolution of inflammation will be evaluated for the treatment of human periodontitis to test a widely discussed hypothesis that host modulation therapy is a more rational target for pharmacotherapy in periodontitis than antimicrobial therapy. In the past, we have not had the tools or molecules to test this hypothesis; we have them now.

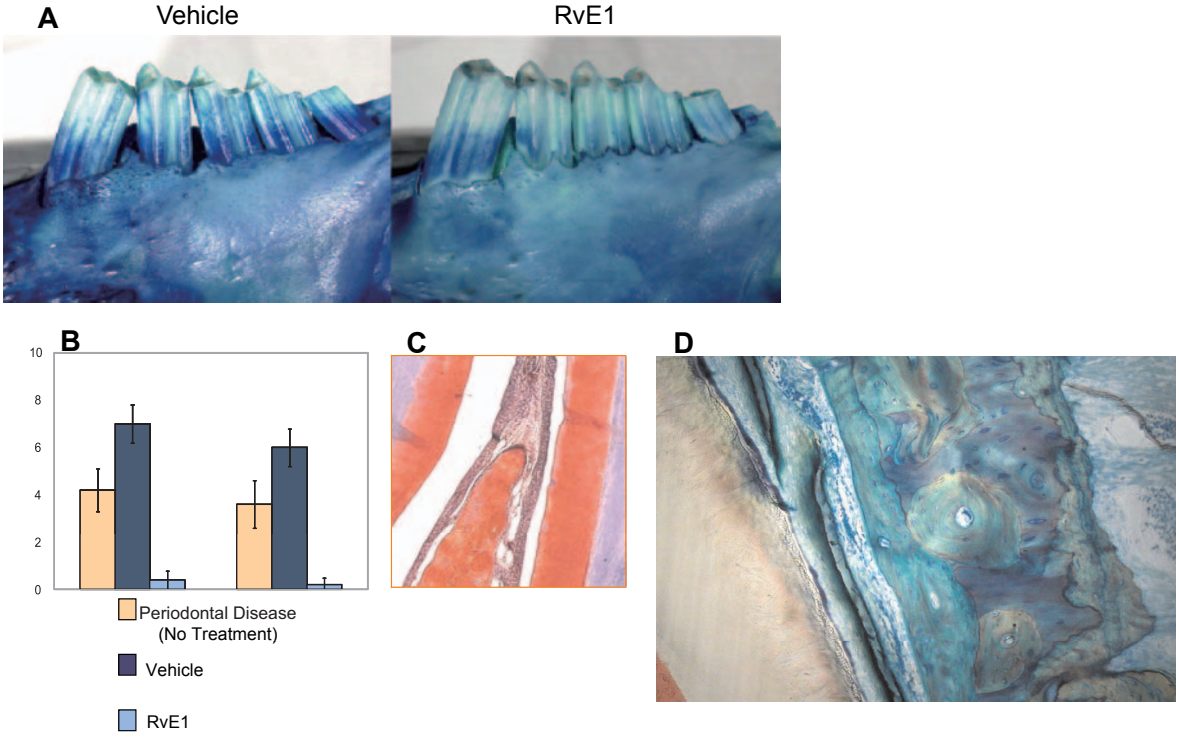

Figure 1. Treatment of Rabbit Periodontitis with RvE1
Chronic periodontitis was treated with topical application of a 1 mg/ml solution of RvE1. A. Clinical images of defleshed alveolar bone reveal regeneration of all lost one compared to vehicle control. B. Quantitative measurement of Bone loss in mm reveals significant bone loss at baseline and after vehicle treatment with no bone loss after RvE1 treatment. C. Masson Trichrome stain reveals histologic evidence of new bone and soft tissue. D. Undecalcified sections reveal new cementum, new periodontal ligament and new bone.

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Liver Immunity and Surgery

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Key words: Liver transplantation, Liver sinusoidal endothelial cells, NK cells, Innate immunity, Immune-therapy, Hepatocellular carcinoma, Viral hepatitis

ABSTRACT

It has been demonstrated that liver sinusoidal endothelial cells (LSECs), which constitute the lining of the hepatic sinusoid, are able to present soluble exogenous antigens to T cells having transgenic T cell receptors. We have reported that LSECs are capable of regulating polyclonal populations of T cells with allo-specificity through direct and indirect antigen recognitions. Enhancing such a tolerogenecity of LSECs might lead to be a novel means to promote acceptance of transplant livers. NK cells are believed to constitute the first line of defense against invading infectious microbes and neoplastic cells by exerting an effector function independent of priming. We have determined the functional properties of peripheral blood NK cells and liver NK cells extracted from liver perfusates of donors and recipients in clinical liver transplantation. Based on the results of these studies, we propose a novel concept to prevent recurrence of hepatocellular carcinoma after liver transplantation, i.e. adoptive transfer of IL-2 stimulated NK cells extracted from donor liver graft perfusate could mount an anti-tumor response without causing toxicity against one-haplotype identical recipient intact tissues.

INTRODUCTION

Premises for the subspecialty of hepatoimmunology include the recognition that the liver is an immune-regulatory organ with unique immunological properties. These properties ensure efficient innate defense against intestinal microbes and toxins and confer a particular capacity for induction of tolerance. Elucidation of such characters of liver-resident immune-regulatory cells might lead to the establishment of novel strategies to prevent/alleviate liver damages during/after liver surgery. Our research efforts to understanding immunological properties of liver sinusoidal endothelial cells (LSECs) and natural killer (NK) cells have been reported in this article.

LSECs TOLERIZE T CELLS ACROSS MAJOR HISTOCOMPATIBILITY COMPLEX BARRIERS

Liver allografts are extraordinarily tolerogenic, and stable grafts can be maintained without immunosuppression in some species. In addition, the presence of a liver allograft can suppress the rejection of other solid tissue grafts from the same donor. The high capacity of the transplanted liver to establish tolerance in an allogeneic host has been attributed to the unique features and architecture of hepatic constituent cells (HCs). However, the details of mechanisms underlying such tolerance state remain to be elucidated.

LSECs, which constitute the lining of the hepatic sinusoid, are also able to present soluble exogenous antigens (Ags) to T cells having transgenic T cell receptors (Knolle, 1999; Limmer, 2000). We investigated the tolerogenicity of LSECs in mice, in which liver allografts are normally accepted without recipient immune suppression across MHC barriers (Onoe, 2006). Through the use of a mixed hepatic constituent cell-lymphocyte reaction (MHLR) assay and transendothelial migration assay, we have demonstrated a novel and surprising effect of LSECs, i.e. naive allogeneic LSECs selectively render reactive $CD4^+$ and $CD8^+$ T cells tolerant at least in part via Fas/Fas ligand (FasL) pathway. This result provides the first demonstration that LSECs are capable of regulating a polyclonal population of T cells with certain specificity through direct Ag recognition. The outline of this study is described as following.

Although livers transplanted across MHC barriers in mice are normally accepted without recipient immune suppression, the underlying mechanisms remain to be clarified. To identify the cell type that contributes to induction of such a tolerance state, we established a MHLR assay (Onoe, 2006). Irradiated C57BL/6 (B6) or Balb/c mouse HCs and CFSE-labeled B6 splenocytes were co-cultured. In allogeneic MHLR, whole HCs did not promote T cell proliferation. When LSECs were depleted from HC-stimulators, allogeneic MHLR resulted in marked proliferation of reactive $CD4^+$ and $CD8^+$ T cells. To test tolerizing capacity of the LSECs toward alloreactive T cells, B6 splenocytes that had transmigrated through monolayer of B6, Balb/c or SJL/j LSECs were restimulated with irradiated Balb/c splenocytes (Fig. 1). Non-responsiveness of T cells that had transmigrated through allogeneic Balb/c LSECs and marked proliferation of T cells transmigrated through syngeneic B6 or third-party SJL/j LSECs were observed after the restimu-

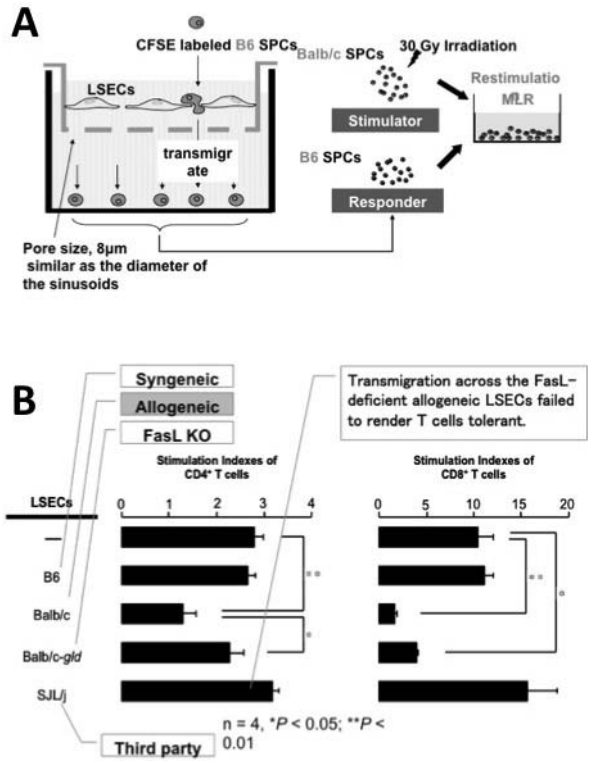

Figure 1. T cells that transmigrated across allogeneic LSECs are rendered specific tolerant to alloantigens by the mechanism involving Fas-FasL pathway. CFSE-labeled non-adherent B6 lymphocytes that transmigrated across LSECs monolayer from various strain mice were subsequently stimulated with irradiated Balb/c splenocytes. **A**, Schemas of transendothelial migration assay are shown. CFSE-labeled non-adherent lymphocytes (10×10^6 cells) from B6 mice were added into each insert and were left for 12 h to migrate through the monolayer of various LSECs. The migrated lymphocytes were co-cultured with irradiated (30 Gy) splenocytes from Balb/c mice in subsequent MLR. LSECs from B6, Balb/c, Balb/c-*gld*, or SJL/j mice were used to form a monolayer. **B**, Stimulation indexes of alloreactive T cells in the subsequent MLR using transmigrated B6 lymphocytes as responder and irradiated Balb/c splenocytes as stimulator are shown. Means ± SEM of four independent experiments are shown. $^*P < 0.05$; $^{**}P < 0.01$. (Onoe, T., et al. 2006).

lation. Transmigration across the Fas ligand-deficient Balb/c LSECs failed to render $CD4^+$ T cells tolerant. Thus, we demonstrate that FasL expressed on naïve LSECs can impart their tolerogenic potential upon alloantigen recognition via the direct pathway. This presents a novel relevant mechanism of liver allograft tolerance; i.e. LSECs are capable of regulating a polyclonal population of T cells with direct allospecificity, and Fas/FasL pathway is involved in such LSECs-mediated T cell regulation (Fig. 2).

We further established an *in vivo* model for evaluating the immunomodulatory effects of allogeneic LSECs on corresponding T cells (Banshodani, 2012). Allogeneic BALB/cA LSECs were injected intraportally into recombination activating gene 2 gamma-chain double-knockout (RAG2/gc-KO, H-2^b) mice lacking T, B, and NK cells. In order to facilitate LSEC engraftment, the RAG2/gc-KO

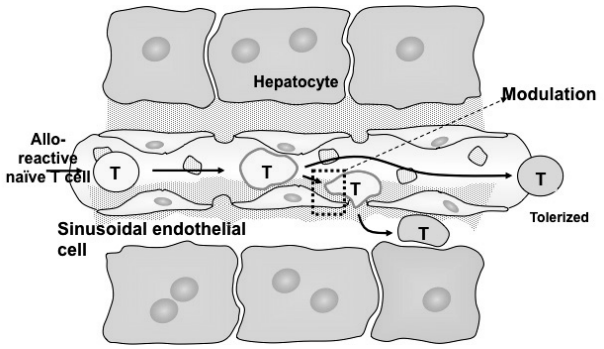

Figure 2. LSECs have tolerogeneic property. Circulating leukocytes are forced into frequent contact with LSECs owing to the small diameter of the sinusoids. During cell-cell contact in the sinusoidal lumen, alloreactive T cells are recognized donor-type MHC expressed on LSECs. Then FasL expressed on the LSECs induced those T cells tolerant. Such sinusoidal architecture likely promotes the immunomodulatory activity of LSECs toward T cells.

mice were injected intraperitoneally with monocrotaline 2 days before the adoptive transfer of LSECs; this impaired the host-LSECs, conferring a proliferative advantage to the transplanted LSECs. After orthotopic allogeneic LSEC engraftment, the RAG2/gc-KO mice were immune reconstituted intravenously with C57BL/6 splenocytes. After immune reconstitution, mixed lymphocyte reaction (MLR) assay using splenocytes from the recipients revealed that specific inhibition of host $CD4^+$ and $CD8^+$ T cell proliferation was greater in response to allostimulation with irradiated BALB/cA splenocytes rather than stimulation with irradiated third party SJL/jorllco splenocytes. This inhibitory effect was attenuated by administering anti-programmed death ligand 1 (PD-L1) monoclonal antibody during immune reconstitution in the above-mentioned mice, but not in RAG2/gc-KO mice engrafted with FasL-deficient BALB/cA LSECs. Furthermore, engraftment of allogeneic BALB/cA LSECs significantly prolonged the survival of subsequently grafted cognate allogeneic BALB/cA hearts in RAG2/gc-KO mice immune reconstituted with bone marrow transplantation from C57BL/6 mice. Thus, murine LSECs have been proven capable of suppressing T cells with cognate specificity for LSECs in an *in vivo* model. The programmed death 1/PD-L1 pathway is likely involved in these suppressive effects.

LSECs CAPTURING ALLOGENEIC CELLS TOLERIZE T CELLS WITH INDIRECT ALLOSPECIFICITY

Although it is known that portal venous injection (PI) of allogeneic donor cells leads to tolerance to the subsequently transplanted allografts, the detailed mechanism remains unclear. We have demonstrate that the indirect pathway of alloantigen presentation via LSECs is involved in allospecific T cell tolerance induced by PI of donor-type splenocytes (Tokita, 2006). To eliminate the direct $CD4^+$ T cell response, B6 MHC class II-deficient *C2ta*^{tm1Ccum} (C2D) mice were used as donors. PI of irradiated B6 C2D splenocytes into Balb/c mice lead to the

indefinite acceptance of subsequently grafted B6 C2D hearts. The Balb/c LSECs that endocytosed B6 C2D splenocytes following treatment with PI expressed MHC class II and FasL. By transmigration across the LSECs from Balb/c mice treated with PI of B6 C2D splenocytes, naive Balb/c $CD4^+$ T cells lost responsiveness to stimulus of Balb/c splenic APCs capturing donor-type B6 C2D alloantigens, while maintaining a normal response to stimulus of Balb/c splenic APCs capturing third-party C3H alloantigens. The transmigration of naive Balb/c $CD4^+$ T cells across the LSECs from Balb/c FasL-deficient mice treated with PI of B6 C2D splenocytes failed to induce such a tolerance state. Thus, T cells with indirect allospecificity were rendered tolerant to alloantigens by contact with autologous LSECs that captured allogeneic cells, at least in part via the Fas/FasL pathway. We have further demonstrated that invariant natural killer T (NKT) cells plays a significant role in such immunosuppressive effects induced by LSECs (Shishida, 2008). The endocytic activity of LSECs toward intraportally injected splenocytes from B6 MHC class II-deficient *C2ta*^{tm1Ccum} (C2D) mice was markedly impaired in BALB/c CD1d-deficient (*CD1d*$^{-/-}$) mice. The intraportal adoptive transfer of LSECs isolated from BALB/c wild-type mice treated with a portal injection of B6 C2D splenocytes into BALB/c mice significantly prolonged the survival of subsequently transplanted heart allografts; however, the transfer of LSECs isolated from similarly treated BALB/c *CD1d*$^{-/-}$ mice did not produce such a survival prolonging effect. These findings indicate that NKT cells are required for the LSEC-induced immune modulation of T cells with indirect allospecificity.

DIFFERENCE IN CYTOTOXICITY AGAINST HEPATOCELLULAR CARCINOMA (HCC) BETWEEN LIVER AND PERIPHERY NK CELLS IN HUMAN

NK cells are thought to provide a first line of defense against invading infectious microbes and neoplastic cells by exerting an effector function without the necessity for priming. Given the efficacy of NK cells in selectively killing abnormal cells, a variety of approaches have been taken to try and selectively augment NK cell response to tumors. The adoptive transfer of NK cells demonstrates the ability of NK cells to mount a therapeutic anti-tumor response and suggests that NK cells can be utilized in controlling human malignancy (Leung, 2004; Meller, 2004). In these studies, autologous or even haploidentical lymphokine-activated killer cells obtained from peripheral blood mononuclear cells (PBMCs) have been administered to patients, although their comprehensive role in the treatment of selected malignancies remains to be elucidated.

It has been known that NK cells are quite abundant in the liver of mice, in contrast to a relatively small percentage in the peripheral lymphatics. The underlying reason for this anatomically biased distribution has not been fully elucidated. In addition, liver NK cells have been shown to mediate higher cytotoxic activity against tumor cells than spleen or peripheral blood (PB) NK cells in rodents. However, such differences between liver and PB NK cells have not been extensively investigated in

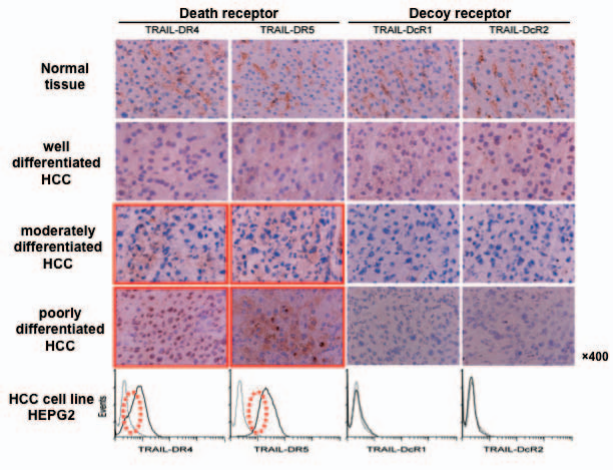

Figure 3. Differential expression of TRAIL receptors in normal liver tissue and HCC tissue.
Immunohistochemical expression of TRAIL-DR4, -DR5, -DcR1 and -DcR2 in normal liver tissue, tumor site of well differentiated HCCs, moderately differentiated HCCs and poorly differentiated HCCs. Magnification: × 400. Immunopathological findings shown are representative of three individual samples in each categorized HCCs. Surface expression of TRAIL receptors on the surface of HepG2 was analyzed by FCM. Dotted lines represent negative control staining with isotype-matched mAbs. HepG2 expressed high TRAIL-DR4 and -DR5 but no TRAIL-DcR1 and DcR2, resembling poorly differentiated HCCs. (Ishiyama, K., et al 2006).

human because of the limited availability of appropriate human samples. We have determined phenotypic and functional properties of liver NK cells extracted from donor and recipient liver perfusates in clinical living donor liver transplantation (LDLT) (Ishiyama, 2006). Donor liver NK cells showed the most vigorous cytotoxicity against a HCC cell line after *in vitro* IL-2 stimulation, compared with donor and recipient PB NK cells and recipient liver NK cells. IL-2 stimulation lead to an increased expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) on liver NK cells, which has been shown to be critical for NK cell-mediated anti-tumor cell killing without affecting normal cells. In addition, we have confirmed that HCC expressed the death-inducing TRAIL receptors (TRAIL-Rs), TRAIL-R1/death receptor (DR) 4 and TRAIL-R2/DR5 that contain cytoplasmic death domains and signal apoptosis (Fig. 3). These findings raise a novel concept to prevent recurrence of HCC after liver transplantation, i.e. adoptive transfer of IL-2 stimulated NK cells extracted from donor liver graft into one-haplotype identical recipients.

ADOPTIVE IMMUNOTHERAPY WITH LIVER DERIVED NK CELLS SHOWS ANTI-HCC AND ANTI-HCV ACTIVITY AFTER LIVER TRANSPLANTATION

Antitumor activity of liver NK cells reportedly decreases after partial hepatectomy, suggesting that patients with such depressed immune status are susceptible to HCC recurrence after partial hepatectomy or par-

tial liver transplantation. Therefore, adoptive immunotherapy using activated NK cells probably has potential as a strategy to reconstitute the depressed immune status in HCC patients after partial liver transplantation. Using mice as a model, we investigated the influence of partial hepatectomy on NK cell activity against HCC and the potential of adoptively transferring activated NK cells to prevent HCC recurrence after partial hepatectomy (Ohira, 2006). Intraportal injection of $1–5 \times 10^6$ Hepa1-6 cells (hepatoma cell line) did not result in liver metastases in untreated B6 mice, but led to the growth of liver metastases after extensive partial hepatectomy. Utilizing this murine HCC metastasis model, we investigated the antitumor activity of both remnant liver and exogenously transferred NK cells. The anti-HCC activity of liver NK cells significantly decreased after partial hepatectomy. The expression of CD69 and TRAIL on liver NK cells was temporarily downregulated. The adoptive transfer of NK cells, including a TRAIL-expressing fraction, extracted from the liver perfusates of poly I:C-stimulated B6 mice inhibited the growth of liver metastasis in B6 or (B6 × BALB/c) F1 (B6CF1) mice that underwent hepatectomy and received intraportal Hepa1-6 injection (Fig. 4). These findings indicate that adoptive immunotherapy using activated NK cells extracted from normal liver perfusates may be a novel technique for reconstituting the depressed immune status in cases of living donor liver transplantation involving HCC patients, recipients of a partial liver graft.

Liver failure and HCC due to chronic hepatitis C infection are the most common indications for liver transplantation (LT), and the incidences of both have been projected to increase further in the future. Recurrent HCV infection of the allograft is universal, occurs immediately after LT, and is associated with accelerated progression to cirrhosis, graft loss, and death. This reflects the suppression of those host-effector immune responses that usually control HCV replication, suggesting that the immunosuppressive environment may play a major role in the rapid progression of recurrent HCV infection after LT. Further, the immunosuppressive condition described above is considered to increase the incidence of cancer recurrence after LT in HCC patients. As described above, we proposed the novel strategy of adjuvant immunotherapy for preventing the recurrence of HCC after LT; this immunotherapy involves intravenously injecting LT recipients with activated liver allograft-derived NK cells (Ishiyama, 2006; Ohira, 2006). Since the immunosuppressive regimen currently used after LT reduces the adaptive immune components but effectively maintains the innate components of cellular immunity, the augmentation of the NK cell response, which is thought to play a pivotal role in innate immunity, may be a promising immunotherapeutic approach. We confirmed that the IL-2/anti-CD3 mAb (OKT3)-treated liver allograft-derived NK cells expressed a significantly high level of the TRAIL, which is a critical molecule for tumor cell killing. Further, these cells showed high cytotoxicity against HCC cells, with no such effect on normal cells. After obtaining approval from the ethical committee of our institute, we successfully administered adoptive immunotherapy with IL-2/OKT3-treated liver lymphocytes to liver cirrhosis patients with HCC in a phase I trial (Fig. 5). Although the long-term benefits of this approach with regard to the control of HCC recurrence after LT remain to be elucidated, this trial provided a unique opportunity to study whether the adoptive administration of IL-2/OKT3-treated liver lymphocytes could also mount an anti-HCV response in HCV-infected LT recipients. We have demonstrated for the first time

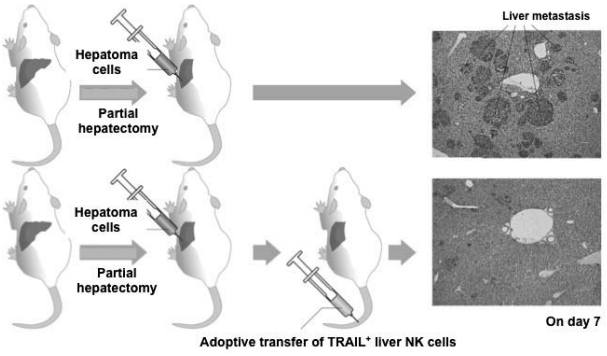

Figure 4. The adoptive transfer of activated liver NK cells inhibited the growth of liver metastasis induced by a portal venous injection of hepatoma cells in extensively hepatectomized mice. Representative histopathological findings of the liver specimen (H.E., ×4 objective). Specimens from the untreated, partially hepatectomized (PHx), and NK cell-receiving groups were inoculated after partial hepatectomy. The adoptive transfer of activated B6 liver NK cells inhibited the growth of liver metastasis induced by a portal venous injection of hepatoma cells in extensively hepatectomized B6 mice. B6 mice were that either underwent or did not undergo partial hepatectomy and injected with tumor cells on Day 0. On Day 3, the NK inoculation group was administered an intravenous injection of 5×10^5 B6 liver NK cells. (Ohira, M., et al 2006).

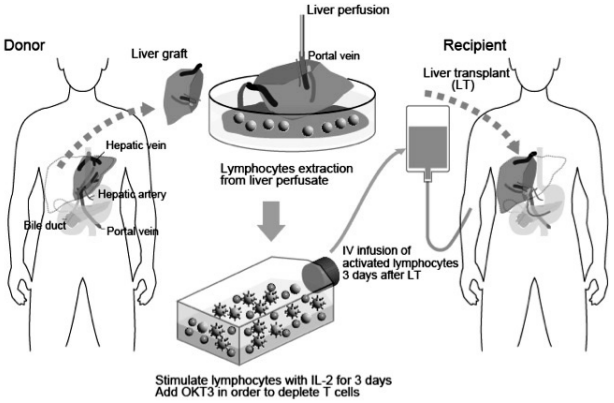

Figure 5. Schematic outline of adoptive immunotherapy with lymphocytes extracted from liver allograft perfusate. The therapy involved giving an intravenous injection of IL-2/OKT3-treated liver lymphocytes to LT recipients. The lymphocytes were extracted from the donor liver graft perfusate. After 3 days of culture with IL-2 (100 JRU/mL), the activated liver NK cell-enriched lymphocytes were administered to the LT recipients through venous circulation. OKT3 (1 μg/mL) was added to the culture medium 1 day before this administration in order to prevent GVHD. (Ohira, M., et al 2009)

that adoptive immunotherapy with IL-2/OKT3-treated liver lymphocytes, including abundant NK and NKT cells, shows anti-HCV activity after LT even in an immunosuppressive environment (Ohira, 2009). After obtaining approval from the FDA in USA, we commenced the similar phase I trial among the cadaveric LT recipients at Miami University (Ohira, 2012).

DISCUSSION

We have demonstrated immunoregulatory effects of LSECs on T cells with direct allospecificity beyond MHC barriers using an *in vitro* mixed LSEC-lymphocyte co-culture model (Onoe, 2005). In that model, cell-cell contact was necessary to induce the inhibitory effects of LSECs on alloreactive T-cell proliferation. *In vivo*, the cumulative surface area of LSECs is very large, and hepatic microcirculatory parameters allow frequent contact between LSECs and passenger leukocytes. Considering the large volume of blood that passes through the liver daily, it is probable that LSECs are ideally positioned within the liver to regulate alloimmune responses. We further investigated the immunoregulatory effects of LSECs on alloreactive T cells in an *in vivo* model in which exogenously inoculated allogeneic LSECs were engrafted orthotopically on the liver sinusoidal endothelium (Banshodani, 2012). The possible mechanisms for LSEC-induced suppressive immune regulation specifically on allogeneic T cells might be associated with the death-inducing molecules that are constitutively expressed on LSECs (*e.g.*, FasL, PD-L1). In addition to the death-inducing molecules, regulatory T cells (Tregs) might also play a role in LSEC-induced suppression of allogeneic T cells. It has been demonstrated that LSECs prime $CD4^+$ T cells to a $CD45RB^{low}$ memory phenotype lacking marker cytokine production for effector cells and that those T cells functionally belong to the $CD25^{low}$ $FoxP3^-$ Tregs family. Those LSEC-primed Tregs are thought to contribute to shifting antigen-dependent immune responses to tolerance toward exogenous antigens or endogenous self-antigens. A similar mechanism might be involved in the immunosuppressive effects of LSECs toward the alloreactive T cells. Nevertheless, we have proven that allogeneic LSECs are capable of suppressing T cells with specificity cognate to the LSECs in both of *in vitro and in vivo* murine models. These results sggest that immunosuppressive therapy can be minimized after allogeneic LT under the reliable immune-monitoring, leading to the achivement of immue-tolerance state.

Lymphokine-activated killer (LAK) cells for immunotherapy are conventionally generated following expansion in the presence of IL-2 for a relatively short culture period. The heterogeneous LAK cell population consists of non-major histocompatibility complex-restricted CD3•CD56• and CD3•CD56• cell subsets, both of which contribute to the cytolytic property of LAK cells. The unique CD3•CD56• cells are generally referred to as NK-like T cells, because, similar to NK cells, they do not require prior specific sensitization to induce recognition of target cells. Addition of anti-CD3 mAb at the initiation of culture, prolongation of culture duration, and addition of various stimuli at the end of culture are improved methodologies to culture LAK cells and reportedly result in better expansion over the original described method. Such expanded LAK cells have clinically demonstrated modest efficacy against metastatic renal cell carcinoma and melanoma. The clinical efficacy of adoptive immunotherapy with IL-2 and anti-CD3 mAb-induced LAK cells has been also proved in terms of prolongation of relapse-free survival for patients with HCC following resection of the primary tumor, although the details of the mechanisms underlying such effects remain unclear (Takayama, 2000).

We have demonstrated that CD56• NK cells can be extracted from the liver allograft perfusate during transplant surgery, and short culture with IL-2 and anti-CD3 mAb induces the anti-HCV activity as well as the anti-HCC activity of the NK and NK-like T cells (Ohira, 2009). Short-term (three days) stimulation with IL-2 significantly up-regulates the expression of TRAIL on liver NK cells, but this effect is barely observed on NK cells from PBMCs. Molecular cloning of TRAIL -receptors elucidated that TRAIL binds to at least four receptors: two are death-inducing receptors (TRAIL-R1/DR4 and TRAIL-R2/DR5), containing cytoplasmic death domains and mediate signal apoptosis; the other two are death-inhibitory receptors (TRAIL-R3/DcR1 and TRAIL-R4/DcR2), lacking a functional death domain and do not mediate apoptosis. However, all have similar affinities and the latter pair may act as decoys.

In addition to anti-neoplastic effects, adoptive immunotherapy with LAK cells may lead to viral clearance. In fact, a reduction in hepatitis B virus (HBV) load has been described in patients undergoing treatment with LAK cells. LAK cells might suppress HBV replication through the secretion of IFN-γ and TNF-α. Despite such an attractive approach, this therapy has never been applied to suppress HCV replication. In general, in the early phase of viral infection, the first line of host defense may be effective in removing the virus; however, recent reports have indicated that HCV effectively escapes the innate immune system comprising NK and NKT cells, resulting in persistent infection. It has been also reported that cross-linking of CD81 on NK cells by the major envelope protein of HCV, HCV-E2, blocks NK cell activation, IFN-γ production, cytotoxic granule release, and proliferation. Engagement of CD81 on NK cells blocks tyrosine phosphorylation through a mechanism that is distinct from the negative signaling pathways associated with NK cell inhibitory receptors for major histocompatibility complex class I molecules. These findings prove that HCV-E2-mediated inhibition of NK cells is an efficient HCV evasion strategy, which involves targeting the early antiviral activities of NK cells and allowing the virus to establish itself as a chronic infection. We explored whether CD81 cross-linking-induced inhibitory effects occur even in IL-2-stimulated NK cells. CD81 cross-linking by a mAb specific for CD81 inhibited anti-tumor cytotoxicity and anti-HCV activity mediated by resting NK cells, but this manipulation did not alter both these activities of IL-2-stimulated NK cells. This indicated that exposure to IL-2 before CD81 cross-linking abrogates subsequent inhibitory signals in NK cells and encourages us to study the possibility of adoptive immunotherapy with LAK cells to inhibit HCV replication. We have

demonstrated that CD56• NK cells derived from liver resident lymphocytes display anti-HCV activity after short-term culture with IL-2 and anti-CD3 mAb through the secretion of IFN-γ (Ohira, 2009). Similarly, long-term cultivation in the presence of IL-2 and anti-CD3 mAb promotes the inhibitory effects of LAK cells from PBMCs on HCV replication (Doskali, 2011). In conclusions, the immunotherapy with IL-2/anti-CD3 mAb-treated liver allograft-derived NK cells promotes host innate immunity, leading to anti-HCC, and anti-HCV effects after LT.

ACKNOWLEDGMENT & CONFLICTS OF INTEREST

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Latest Facts and Issues about Dental Education in Japan

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1. FIRST REPORT FROM THE DENTAL EDUCATION IMPROVEMENT STUDY COUNCIL

The Dental Education Improvement Study Council, which was established under Ministry of Education, Culture, Sports, Science and Technology Japan (MEXT) in July 2008, compiled its first report in January 2009, based on discussions on what constitutes adequate education to develop dentists with reliable clinical competency.

The report summarizes issues and proposed solutions regarding the following four focus areas:

1) Help students develop clinical competency required for dentists.

<Issues>

- Few academic institutions have defined clear academic goals to be achieved and conducted proper academic performance assessments.
- A decreasing number of hours spent on clinical training due to difficulty gaining patient cooperation and an increasing number of hours spent preparing for the national examination.

<Solutions>

- Specify the number of credits for clinical training and clarify academic goals to be achieved before graduation and required clinical training units.
- Administer the Objective Structured Clinical Examination (OSCE) at each academic institution after clinical training.
- Encourage academic institutions to conduct their clinical training other than the institutions.

2) Provide systematic dental education to produce excellent dentists.

<Issues>

- Less clear distinctions in education among academic institutions.
- Because of the timing of common achievement tests (OSCE and computer-based testing [CBT]), the period of clinical training separate from lecture in classroom.

<Solutions>

- Ensure that each academic institution devises a systematic curriculum and strictly conducts academic performance assessments and promotion evaluations.
- Review the model core curriculum for dental education.
- Introduce a third-party evaluation system to ensure the quality of dental education.

3) Strive to enroll a sufficient number of excellent students who will meet future social needs for dentists.

<Issues>

- Entrance examinations are beginning to lose their function as screening exam at more and more academic institutions.
- The dental career is becoming less attractive due to a surplus of dentists.

<Solutions>

- Specify admission policies and publicize entrance examination information.
- Each academic institution needs to assess students' aptitude and other personal attributes programs; interviews, cooperation from students' high schools, through innovative admission and so on.
- Academic institutions with problems, such as difficulty attracting excellent students and having a low pass rate for the national examination, need to review their student enrollment limit.

4) Produce researchers who will contribute to the bright future of dental dentistry.

<Issues>

- Need the research that integrates basic and clinical aspects, etc.
- Need to start developing research-oriented minds at the undergraduate level.

<Solutions>

- Create more opportunities for students to engage in research at the undergraduate level.
- Dental graduate schools need to clarify their objectives and what to teach according to their clinical dentist/researcher development plans.
- Create a center for producing internationally-competent young researchers beyond the boundaries of academic institutions.

2. REVISION OF MODEL CORE CURRICULUM FOR DENTAL EDUCATION

The model core curriculum for dental education was established in March 2001 as dental education guidelines of the minimum requirements of what dental students should learn before graduation (partially revised in December 2007).

In terms of improving clinical training programs and developing research-oriented minds, this model core cur-

riculum was revised again in March 2011 based on recommendations in the first report from the Dental Education Improvement Study Council.

3. RESULTS OF FISCAL 2012 FOLLOW-UP SURVEY BASED ON THE FIRST REPORT FROM THE DENTAL EDUCATION IMPROVEMENT STUDY COUNCIL

The Dental Education Improvement Study Council conducted follow-up surveys based on its first report and compiled the results into reports in May 2011 and December 2012.

The report on the results of the follow-up survey released in December 2012 includes the following:

<Overall Trends>

- The recommendations contained in the previous follow-up survey report helped to create an overall trend toward improvement and further improvements are expected in the future.
- Some academic institutions have shown few improvements in response to the recommendations in the first report. Such institutions are urged to reflect seriously on their situation and to take actions, such as improving what they teach, reviewing and strictly controlling their enrollment limit, and striving to enroll more excellent students.

<Issues>

1. Excessive over-enrollment, etc.
2. All academic institutions need to share the importance of their students experiencing the entire treatment procedure during their clinical training.
3. Need to improve clinical training programs, clinical competency evaluation methods, etc.
4. Need to enroll excellent students.
5. Need to improve students' academic performance, to decrease students' grade repeat rate, and to raise each institution's national exam pass rate for students who have completed the minimum years required for graduation.
6. Need to develop researchers.
7. All academic institutions need to publicize essential information about their education.
8. Each faculty of dentistry must offer its own unique education.

4. REPORT ON THE DIRECTION OF MEASURES FOR IMPROVING THE QUALITY OF DENTAL EDUCATION

The Dental Education Improvement Study Council compiled a report in December 2012 on the direction of measures for improving the quality of dental education.

The report recommended the following seven measures that could improve the quality of dental education.

1. Conduct follow-up surveys regarding improvement of dental education.
2. Promote to clinical training programs.
3. Introduce mutual evaluations in clinical training on a trial basis.
4. Develop dentists who can meet diverse needs for dental treatment.
5. Improve the common achievement tests conducted before clinical training.
6. Publicize essential information about each academic institution's education on MEXT's website.
7. Introduce a dental education accreditation system on a trial basis.

5. INTRODUCTION OF A DENTAL EDUCATION ACCREDITATION SYSTEM

MEXT began implementing a dental education accreditation system development program in fiscal 2012 to prove that Japanese faculty of dentistry can provide quality education at international standards and to produce excellent globally-competent dentists whose ability exceed international standards.

Tokyo Medical and Dental University and other academic institutions, which have been selected through an open application process under the program, are now conducting a study to help establish a dental education accreditation system at an international level.

In fiscal 2012 a study on overseas accreditation standards and processes was conducted, and in fiscal 2013 Japanese accreditation standards will be established and a Japanese accreditation system will be introduced on a trial basis.

The G60S Connexin 43 mutation is dominant-negative for gap junction formation and function but activating for the osteoblast lineage

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BACKGROUND

Gap junctions and hemichannels mediate cellular communication by allowing the passage of small molecules and ions (e.g. ATP, Ca^{2+}, IP3, cAMP) directly between cells and between cells and their extracellular environment, respectively. Connexin 43 (Cx43), one member of the connexin protein family, is the major gap junction protein found in bone, expressed in osteoblasts, osteocytes, osteoclasts and bone marrow stromal cells. Using a genome-wide ENU-mutagenesis screen, we isolated an osteopenic mutant mouse line, *Gja1*^{Jrt}/+, with a G60S mutation in Cx43 that is dominant negative for gap junction formation and function (Flenniken et al., 2005).

RESULTS

Similarly to what is observed in other Cx43 mutant mouse models reported to date, including a global Cx43 deletion, four skeletal cell conditional-deletion mutants and a Cx43 missense mutant (G138R/+), we found that reduced Cx43 gap junction function resulted in mice with early onset osteopenia. However, in contrast to other Cx43 mutants, *Gja1*^{Jrt}/+ mice exhibited both higher bone marrow stromal osteoprogenitor numbers and increased appendicular skeleton osteoblast activity leading to cell autonomous upregulation of both matrix bone sialoprotein (BSP) and membrane-bound receptor activator of nuclear factor kappa-B ligand (mbRANKL). In younger *Gja1*^{Jrt}/+ mice, high BSP along with changes in RANKL-osteoprotegerin (OPG) signaling, contributed to increased osteoclast number and activity resulting in early onset osteopenia. In older animals, however, this effect was abrogated by increased OPG and serum alkaline phosphatase (ALP) so that differences in mutant and wild type (WT) bone parameters and mechanical properties lessened or disappeared with age, which abrogated age-related bone loss in older animals (Zappitelli et al., 2013).

Gja1^{Jrt}/+ mice also exhibit a significant and progressive increase in bone marrow atrophy characterized by increased adipocytes evident as early as 7 weeks of age. We are currently assessing whether a common mechanism underlies both the hyperactive osteoblast phenotype and increased adipogenesis in *Gja1*^{Jrt}/+ mice by assessing differential expression of various signaling pathway molecules. Our data suggest that a common pathway is cell autonomously responsible for the hyperactive osteoblast phenotype and cell non-autonomously responsible for increased bone marrow adipogenesis in Gja1^{Jrt}/+ versus WT mice (Zappitelli and Aubin, in preparation).

CONCLUSION

The G60S Cx43 mutation is a loss-of-function mutation for gap junctions but a gain-of-function mutation for osteoblast (cell autonomous) and osteoclast (cell non-autonomous) activity that leads to age-related alterations in bone turnover with early onset osteopenia and abrogation of old age-related bone loss in *Gja1*^{Jrt}/+ mice. Alterations in signaling pathways is at least partly responsible for the cell autonomous hyperactive osteoblast phenotype, and for the cell non-autonomous increased bone marrow adipogenesis in *Gja1*^{Jrt}/+ versus WT mice.

Education Session

Reformation, Standardization and Accreditation of Dental Education

From Mutual Recognition Arrangement (MRA) towards Core Competencies of Dental Professions in ASEAN Economic Community (AEC)

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University of Washington

D.C.N. Chan

From Mutual Recognition Arrangement (MRA) towards Core Competencies of Dental Professions in ASEAN Economic Community (AEC)

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ABSTRACT

ASEAN Mutual Recognition Arrangement (MRA) on Dental Practitioners has led to the identification of Competencies of the Dental Professions in the ASEAN countries. Once the common Competencies have been identified and collected, it could be used by the ASEAN administrative body to employ as a tool towards accreditation and licensing of Dental Practitioners in ASEAN in the future. South East Asia Association for Dental Education (SEAADE) has encouraged its country members to identify each country Competencies of their Dental Profession. The Competencies of the Dental Professions in Thailand and Malaysia approved by their Dental Councils in 2012 have been presented.

I. BACKGROUND OF THE ASEAN MUTUAL RECOGNITION ARRANGEMENT (MRA) ON DENTAL PRACTITIONERS.

At the 12th ASEAN Summit in January 2007, the Leaders affirmed their strong commitment to accelerate the establishment of an ASEAN Community by 2015 as envisioned in the ASEAN Vision 2020. ASEAN will provide an MRA for Dental Practitioners that would strengthen professional capabilities by promoting the flow of relevant information and exchange of expertise, experiences and best practices suited to the specific needs of ASEAN Member States.

The objectives of the MRA are: to facilitate mobility of the dental practitioners within ASEAN, to exchange information and enhance cooperation in respect of mutual recognition of dental practitioners, to promote adoption of best practices on standards and qualifications, to provide opportunities for capacity building and training of dental practitioners.

The 24th Meeting of the Healthcare Services Sectoral Working Group (HSSWG) held on 12-13 May 2010 in Pattaya, Thailand and preceded by the ASEAN Joint Coordinating Committee (AJCCDs) on Nursing, Medical and Dental Practitioners on 11 May 2010.

The AJCCD discussed Thailand paper on Core Competencies for Dental Practitioners.

II. MUTUAL RECOGNITION OF CORE COMPETENCIES AS A TOOL TOWARDS ACCREDITATION AND LICENSING OF DENTAL PRACTITIONERS IN ASEAN.

In the recent past, many accrediting and licensing bodies have implemented competency-based standards requiring schools of dentistry to modify their educational programming from process-related documentation defined by numeric requirements to mastery level expectations and outcomes (Chambers, 1994).

As a result, all schools began transitioning to defining expectations of student learning outcomes and improving measurements to support the evidence of attainment. Schools defined competencies that they expected graduates to attain during the educational program. Curricula were then designed to satisfy the adopted individual school competencies that aligned with the accreditation standards. Perhaps no other external influence had such a far-reaching influence on the state of dental education at the time (Pyle, 2012).

The most significant milepost is the attainment of the first professional degree, which corresponds to the attainment of professional *competency*—the ability to begin independent, unsupervised dental practice. Competencies are abilities essential to beginning the practice of dentistry. The competencies must be supported by working *knowledge* of basic biomedical, behavioral, and clinical sciences and biomaterials; by cognitive and psychomotor *skills*; and by professional and ethical *values*. The integration and application of the basic biomedical sciences are considered a critical element in the development of competencies for the future. These abilities incorporate understanding, skill, and values in an integrated response to the normal range of problems and challenges in the practice of dentistry that will allow a graduate to practice safely and independently. The level of performance requires some degree of speed and accuracy consistent with patient well-being. It also requires an awareness of what constitutes acceptable performance under normal circumstances. Competent practitioners must use these abilities as the basis for clinical decisions and in professional, patient, and public education. They must have a desire for self-improvement. Because competencies are written to describe the performance of graduates in dental settings, as opposed to the performance of students in courses, the development of competencies is an interdisciplinary process (ADEA Competencies for the New Dentist, 2004).

The South East Asian Association for Dental Education (SEAADE) as an Dental Education body has been active in promoting cooperation and progress of Dental Education in the region. The Peer Review Programme is one of many activities SEAADE trying to

bring together for the improvement and raising the standard and quality of the Dental Faculties by friendly visitation to the member institutions and giving feedback or suggestions to the institution visited. Any Best Practices which they have found out from their visitation will be posted on the SEAADE website so that other Dental Faculties or Institutions can also learn from the findings. By this way, we can raise the awareness of the existing standard and quality of the Dental Education in this region ready for the ASEAN Economic Community in 2015. Since 2010 SEAADE has encouraged its member countries to identify competencies of the dental profession agreed upon in their own country so that SEAADE could gather together those competencies and grouping those which are in similarity in the majority countries as Core Competencies. The followings are examples of Competencies of Dental Practitioners in Thailand and Malaysia as approved by the Dental Council of Thailand and Malaysia (2012).

1. Competencies of Dental Practitioners in Thailand

Part I. Professionalism : A dentist must have contemporary knowledge and understanding of the broader issues of dental practice, be competent in a wide range of skills, including research, investigative, analytical, problem-solving, planning, communication, presentation and team skills and understand their relevance in dental practice. Be competent to display appropriate caring behavior towards patients to display appropriate professional behavior towards all members of the dental team. Have knowledge and understanding of the moral and ethical responsibilities involved in the provision of care to individual patients and to populations, and have knowledge of current laws applicable to the practice of dentistry.

Have knowledge of the ethical principles relevant to dentistry and be competent at practicing with personal and professional integrity, honesty and trustworthiness. Have knowledge and understanding of patients' rights, particularly with regard to confidentiality and informed consent, and of patients' obligations, etc.

Part II. Basic Biomedical, Technical & Clinical Sciences : A dentist must have sufficient knowledge and understanding of the basic biomedical, technical and clinical sciences to understand the normal and pathological conditions relevant to dentistry and be competent to apply this information to clinical situations. A dentist must be competent at acquiring and using information and in a critical, scientific and effective manner, and in evaluating published clinical and basic science research and integrate this information to improve the oral health of the patient, etc.

Part III. Clinical Skills : A dentist must be competent in obtaining and recording a comprehensive medical history and a history of the patient's oral and dental state. This will include biological, medical, psychological and social information in order to evaluate the oral condition in patients of all ages. Be competent in performing an appropriate physical examination; interpreting the findings and organizing further investigations, to identify the chief complaint of the patient and obtain a history, producing a patient record and maintain accurate patient treatment record entries, performing an extra and intra oral examination appropriate for the patient, including assessment of vital signs, and record those findings. Be competent at assessing sensory and motor function of the mouth and jaws, salivary function, orofacial pain, facial form and deviations from the normal. Have knowledge and understanding of the scientific principles of sterilization, disinfection and antisepsis to prevent cross-infection in clinical practice. Be competent effectively to prevent and manage the majority of medical and dental emergency situations encountered in the general practice of dentistry and be able to perform basic life support. Be competent in the management of acute pain, haemorrhage, injury and infection of the oral region, etc.

Part IV. Oral Health Promotion : Be competent to holistically evaluate oral health status of individuals, families and groups in the community. Be competent in applying the principles of health promotion and disease prevention to meet the need of the target group. Be competent in understanding the complex interactions between oral health, nutrition, general health, drugs and diseases that can have an impact on oral health care and oral diseases. Have knowledge of the social, cultural and environmental factors, which contribute to health or illness, etc.

2. Competencies of New Dental Graduates

Competency assumes that all decisions, tasks and behaviours carried out are supported by sound knowledge and skills in biomedical, behavioural and clinical dental science and in an ethical and professional manner as spelled out in the Code of Professional Conduct of the Malaysian Dental Council.

Upon graduation, students should have the following outcomes:-

1. Possess scientific knowledge to support the practice of dentistry. (Cognitive)
2. Demonstrate clinical skills to practice dentistry independently. (Psychomotor)
3. Demonstrate teamwork skills in managing oral health care for individuals and community. (Psychomotor & Affective)
4. Display ethical values and professionalism in practicing dentistry within the confines of the laws governing the profession. (Cognitive, Psychomotor & Affective)
5. Communicate effectively with peers in the dental and other health professions, patients and community. (Psychomotor & Affective)
6. Appraise and apply current scientific information and techniques in the practice of dentistry. (Psychomotor)
7. Display skills for lifelong learning and continuing professional development. (Cognitive & Psychomotor)
8. Display entrepreneurial skills in the management of dental practice. (Cognitive & Psychomotor)

THE DENTAL GRADUATE IS EXPECTED TO ACHIEVE THE FOLLOWING COMPETENCIES ON COMPLETION OF A BASIC DENTAL DEGREE PROGRAMME.

Domain 1: Knowledge

PO1: Possess scientific knowledge to support the practice of dentistry.

To be able to explain the interactions between general health, oral health, nutrition, drugs and diseases that can have an impact on dental care, apply the principles of oral health promotion and disease prevention. relate basic structure and functions of the human body at organ, tissue, cellular and molecular levels to the practice of dentistry, explain the aetiology & pathogenesis of systemic conditions & disease processes affecting the human body including orofacial region, distinguish the signs and symptoms of orofacial diseases and related systemic conditions, explain normal and abnormal orofacial development, explain radiographic techniques and radiation safety in the practice of dentistry, select relevant investigative procedures to aid the diagnosis and management of common oral diseases, explain the pharmacotherapeutics of drugs commonly used in dentistry, apply the principles of occlusion and its significance in the management of various orofacial diseases and conditions, select local anaesthetic procedures in the management of pain during dental treatment describe sedation and general anaesthetic procedures in the control of pain related to dentistry, explain cranio-facial form and relationships, including evidence of deviation from the norm, explain the concepts of dento-facial aesthetics and its application, differentiate the principles of restoration and replacement of primary and permanent dentition, identify the treatment needs of various target groups including special needs and geriatrics, apply principles and methods of sterilisation, disinfection and antisepsis to prevent cross-infection in clinical practice, demonstrate the influence of behavioural, social and environmental factors in the delivery of oral health care, justify the selection of dental materials based on the science and applications as well as their limitations and related environmental issues, apply basic principles of exodontia and minor oral surgical procedures, explain the methods of prevention and management of common medical and dental emergencies.

Domain 2: Practical and Clinical Skills

PO2: Demonstrate clinical skills to practice dentistry independently

Demonstrate the prevention methods of common orofacial diseases and conditions based on scientific evidence, demonstrate health promotion skills, adapt appropriate methods of infection control in clinical practice, display the ability to obtain and record relevant medical, dental and social history, perform clinical examinations, intraoral radiographic and other necessary investigations relevant to the practise of dentistry, integrate findings of a comprehensive examination to make a diagnosis, formulate an appropriate treatment plan based on clinical examinations and investigations, perform simple restorative procedures in primary and permanent dentition including pulp management of single rooted teeth, perform complex restorative procedures in primary and permanent dentition including onlays, single crowns, short span bridges and root canal therapy of uncomplicated multirooted teeth, construct simple prostheses for replacement of missing dentition, perform non-surgical management of periodontal conditions, perform Basic Life Support in the management of medical emergencies in dental practice, manipulate commonly used dental materials in dental practice, demonstrate administration of local and topical anaesthesia and management of their potential complications, perform simple oral surgical procedures including exodontia perform simple orthodontic treatment including removable appliances, display the ability to prescribe and advise the use of common pharmaceutical agents related to dentistry.

Domain 3: Social Skills, Teamwork and Responsibility

PO3: Demonstrate teamwork skills in managing oral health care for individuals and community

Display skills in implementing preventive measures for individuals and community according to the risk assessment, perform patient care by taking into consideration their intellectual and socio-emotional characteristics, display ability to engage patient and/or their parents, guardians or care givers in their oral health care, display the ability to lead or contribute as team member.

Domain 4: Values, Ethics, Moral and Professionalism

PO4: Display ethical values and professionalism in practicing dentistry within the confines of the laws governing the profession.

Comprehend the Code of Professional Conduct from the Malaysian Dental Council, comprehend the laws and regulations related to the practice of dentistry in Malaysia, explain the role and function of professional organizations and regulatory bodies, describe the professional duties of care in dentistry in line with the Patients' Charter, follow the requirements for informed consent and confidentiality of patient record, demonstrate ethical values and professional behaviour towards patients, members of the dental team and other health care personnel, recognize the limitations of their clinical skills and refer accordingly.

Domain 5: Communication Skills and Interpersonal Relationships

PO5: Communicate effectively with peers in the dental and other health professions, patients and community.

Display good doctor-patient relationship in the delivery of oral health care, identify patients' expectations, demands, needs and attitude with regards to oral health care, display effective communication with the dental team, patients, and other health care personnel to facilitate the delivery of oral health care, perform an appropriate referral of a patient based on professional judgment.

Domain 6: Critical Thinking & Scientific Skills

PO6: Appraise and apply current scientific information and techniques in the practice of dentistry.

Apply clinical reasoning skills in decision making for oral health care delivery, Apply evidence-based

approach in the practice of dentistry.

Domain 7: Continuing Professional Development and Lifelong Learning

PO7: Display skills for lifelong learning and continuing professional development.

Recognize the resources for lifelong learning, demonstrate ability to acquire knowledge and scientific evidence.

At the 24th SEAADE Annual Scientific Meeting in Bangkok on 19-20 August, 2013, the Council has requested the country members to identify Competencies of the Dental Professions of each country. Indonesia, Cambodia, the Philippines, Myanmar and Vietnam will submit their countries' Competencies in due course.

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New era of Dental Education: Quality assurance of dental education through the accreditation in Korea

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ABSTRACT

More than ever before in the history of mankind, we live in a world that is ever-changing; fully equipped with advanced technology and tremendous international mobility. This new environment demands that educational institutions change their strategies. Dental education must shift its direction from a narrow focus on teaching knowledge and skill, to a broader, more complex notion of dental education that includes the teaching of ethics, and professionalism, and is focused on the competencies of dentistry. To assure the quality of our graduates, we must review and accredit individual school's achievement through fair, robust, and reliable procedures. The Korean Institute of Dental Education and Evaluation (KIDEE) was founded in 2007 to establish new quality assurance procedures throughout the 11 dental schools in Korea. Its first round of accreditation was completed last year. Currently, it is preparing revised standards and procedures, based on the experience of conducting the first round. This includes the realization that it is important to gain public trust through open and fair processes of quality assurance, even in the highly specialized discipline of dentistry education. With public trust, dental education institutions will be able to seize the opportunity to prepare for an ever-changing society and world.

INTRODUCTION

New Era

Today, more than ever before, including the recent past, we live in a world that is in constant change, in response to globalization, economic turbulence, and technological development. Within this ever changing and advancing global environment, the health of many human beings has been greatly improved, including their oral health. This has impacted the health professions, including dentistry and dental education. The challenge for dental education is to ensure that all who are involved-staff and students within faculty-are at the forefront of advancing knowledge and technology. The key question for dental education is:
"How can we best prepare our students for this new and challenging world?"

Education for the future

We have a long-standing tradition in dental education. We have always aimed to apply scientific advances to dental healthcare delivery. We have developed patient-oriented practices, as an essential ingredient of maintaining high standards of health provision. Increasingly, dental health communities are acknowledging the responsibilities of dentists to society as a whole, though we still have to overcome difficulties in putting this into practice. Change is always difficult, but we have got to find ways to provide an appropriate education for health professionals able to maintain, manage, and improve the health of a society. Moreover, dental education for our new world cannot be confined to producing qualified graduates having only professional knowledge and skills; we also have to educate our student's attitudes toward society. This means that we have to change our curriculum. And not just the curriculum, we also need to educate students quite differently than before.

A new dental education for a changing world must include communication skill, ethical awareness, and an appropriate notion of professionalism that is responsive to the needs of patients. But these things cannot be taught in a context of separated and isolated disciplines; they need to be integrated in a curriculum that is founded on a real clinical context. And, moreover, we must ensure that all the educational outcomes of the students are related to the clinical practice of dentistry.

The goal of implementing an outcomes-based education and securing the trust of society cannot be left to institutions working on their own. We need to work together, and for this we need to forge a consensus between all stakeholders and the public, through open discussion. Furthermore, implementing accountability and shared educational outcomes cannot be confined to the aspirations of a single country. Our new "global" society demands that public accountability should be shared throughout the global community. All the resource we have should be shared within and across the global community of educators.

The Global context of quality assurance in Higher education

As a result of economic growth, international mobility is rapidly increasing. This has brought large changes in the global healthcare environment, including dentistry. Patients and dentist move rapidly between countries. The healthcare provision follows them.

In this context, the UNESCO/OECD guidelines (Quality provision in cross-border higher education, 2005, Table 1) aim to support and encourage international cooperation and enhance awareness of the importance

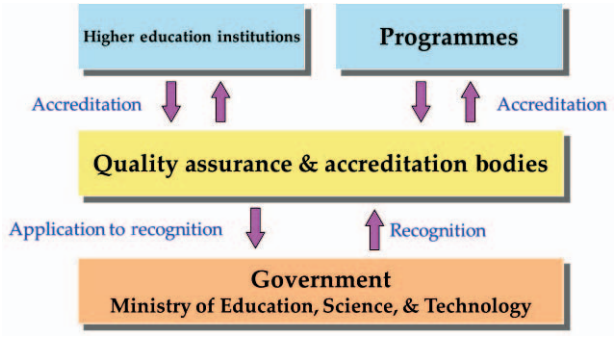

Figure 1. Quality assurance of higher education in Korea

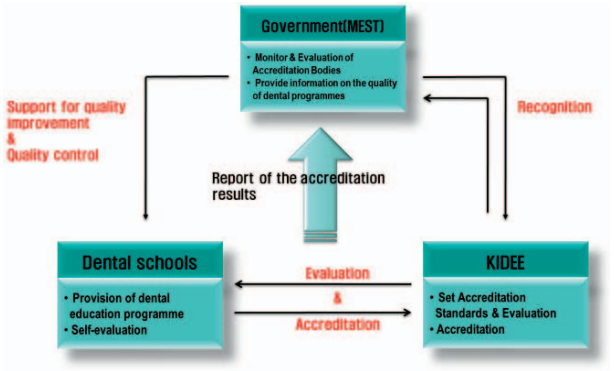

Figure 2. Quality assurance of dental education in Korea

of quality provision in cross-border higher education. The establishment of quality assurance systems has become a necessity, not only for monitoring the quality of higher education delivered within the country, but also the delivery of higher education internationally. This is necessary to protect students and other stakeholders from low-quality and/or disreputable education providers, as well as to encourage the development of quality cross-border higher education that meets human, social, economic and cultural needs.

Quality assurance of Higher education in Korea

Though 'globalization' has become a trendy slogan, it is still more an aspiration than a reality, especially in the Asia-Pacific region. In Korea, dental accreditations were far from globalized, just a decade ago. We had many good reasons to believe that dental education in Korea was up to the standards of best practices elsewhere in the world, but we had no means of communicating this to the rest of the world. We have tried hard to formulate our best practices of dental education in line with global standard. In particular, we have tried to set out our standards within the context of the need for quality assurance (Figure 1).

The role of the Korean Institute of Dental Education and Accreditation (KIDEE) in providing quality assurance of Dental Education in Korea

The Korean Institute of Dental Education & Evaluation (KIDEE) was founded in 2007. Discussion on the necessity of dental education accreditation bodies started in early 2000. In 2004, dental educators formally recognized the need for an Accreditation Body in Dental Education. In 2005, the first report was issued on Accreditation Body of Dental Education with the support of Korean Dental Association. In 2006, a Public Hearing on establishing the Accreditation Body of Dental Education was held, and gained the consensus of the Korean dental education community. In 2007, the Korean Institute of Dental Education and Evaluation (KIDEE) was established, with governmental affirmation.

Though independent of government, it is now in process of recognition as a national accreditation body for dental education by the Korean ministry of Education. The organization also works independently of individual dental education institutions and dental associations in Korea, in carrying out its mission and responsibilities.

Responsibility of KIDEE

The mission of the KIDEE is to advance public oral health in Korea by researching, developing, and applying standards that ensure the quality in dental education and the practice of dentistry. The organization is committed to further supporting the ongoing development of quality assurance measures at both national and international levels, in the context of an increasingly globalized world.

The scope of KIDEE not only relates to accreditation within Korea, but also to setting acceptable standards of practice and theory that can be recognized and accepted globally, across international boundaries (Figure 2). The work and responsibilities of the organization include formulating and approving accreditation standards; evaluating dental programs; determining accreditation status; researching and developing policies on qualifications; and, licensing examinations. In summary, its role is to:

1. Set standards that define quality of education
2. Evaluate & monitor programs for compliance with standards
3. Establish policies & procedures to guide evaluation and decision process
4. Ensure fairness & consistency in process
5. Provide mechanisms for due process
6. Assess own effectiveness

The standards

The standards for program evaluation were first developed in 2008 and these were reviewed after completing a first round pilot study in two dental schools in 2008. A revised version of the standards were completed in 2009, which takes account of the results of that study and sets the findings within the context of international best practice. The core standards developed and used by the KIDEE were divided into five domains: Educational Program, Objectives of Education, Student Policy, Faculty and Staff, and Facilities and Resources (Table 1).

The Accreditation procedure

The purpose of program evaluation and accreditation is to promote excellence in dental education and thus assuring the public that the graduates of accredited dental programs are educated in a core body of knowledge and skills required for independent practice. These stan-

dards therefore provide a framework of basic elements essential to accredited dental education programs, while encouraging flexibility in the ways in which programs pursue excellence.

The accreditation procedure for individual dental school takes one year to complete. The accreditation starts with the submission of a self-study report from the recipient dental school. Review committees were constituted with members from the well-trained reviewer pool. After reviewing the submitted report, a site visit is conducted. A draft report and response are exchanged between the review committee and school. An independent review-decision committee makes the final decisions on the outcomes of the accreditation procedure conducted at the school (Figure 3).

The final decision of the accreditation procedure is categorized into:

- 'Accreditation' (4 years next general review, 2years interim report or visit)
- 'Suspension for 1 year'
- 'No accreditation'

(Figure 4).

From 2009 to 2012 KIDEE successfully completed the accreditation of all 11 dental schools in Korea (Table 2).

Table 1. Standards of institutional evaluation and accreditation

1 Objectives of Education
1.1. Objective-Curriculum Alignment
1.2. Quality Assurance and Improvement
1.3. Application of Institutional Specialty
1.4. Establishment of Competency Criteria
2 Educational Program
2.1. Basic Science
2.2. Clinical Dentistry
2.3. Integration Between Basic Science and Clinical Dentistry
2.4. Humanities and Social Science
2.5. Instruction/Course Evaluation
2.6. Student Assessment
2.7. Environment of Clinical Education
2.8. Implementation of Continuing Education Program
3 Student Policy
3.1. Validity and Objectivity of Admission Policy
3.2. Academic and Career Counseling
3.3. Student Services
3.4. Outcomes Assessment of Graduates
4 Faculty/Staff
4.1. Structure and Distribution of Faculty
4.2. Faculty Evaluation
4.3. Faculty Development
5 Facility and Resources
5.1. Educational Facilities and Equipment
5.2. Research Facilities and Equipment
5.3. Management/Maintenance
6 Institutional Effectiveness
6.1. Purpose/Mission statement of school
6.2. Administration
6.3. Financial Management
6.4. Strategic Goals and Plans

DISCUSSION

This paper began by pointing to the very many changes taking place within the rapidly globalizing world, how this is impacting dental education. In particular, dental education is now equipped with advanced technology and tremendous international mobility. The thrust of this presentation has been that this calls for changes in the way we educate dentists for the future. Even if basic skills and knowledge show some stability, at least for a while, the relationship of dental education to the broader to society is changing rapidly and is likely to continue to change. Society not only expects competent dental practitioners, it also demands that dentist act professionally, ethically, and with enhanced interpersonal communication skills. These and similar competencies need to be built in the dental education curriculum.

Over the past 5-year's experience of dental accreditation in Korea, KIDEE has become aware that even in the professional education of highly specialized discipline such as dental education, it is necessary to gain public trust through open, fair processes of quality assurance. It is the belief and expectation of KIDEE that this will provide dental education institutions in Korea with an appropriate opportunity to prepare dental practitioners who are ready to face an ever-changing society, and with the necessary skills and attitudes to reach out into a rapidly changing world.

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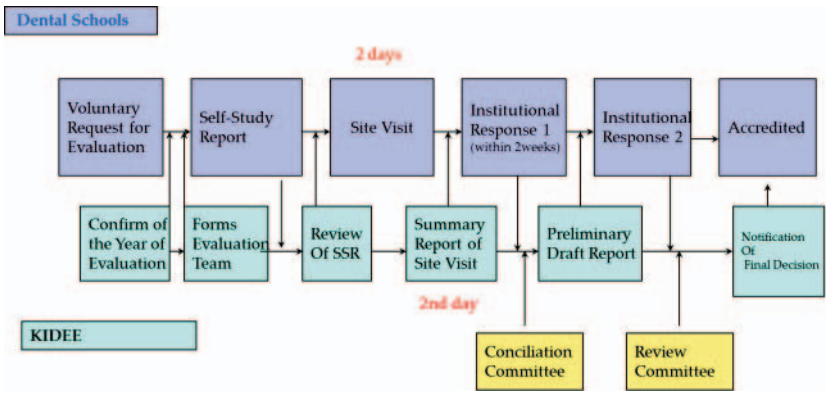

Figure 3. The accreditation procedure

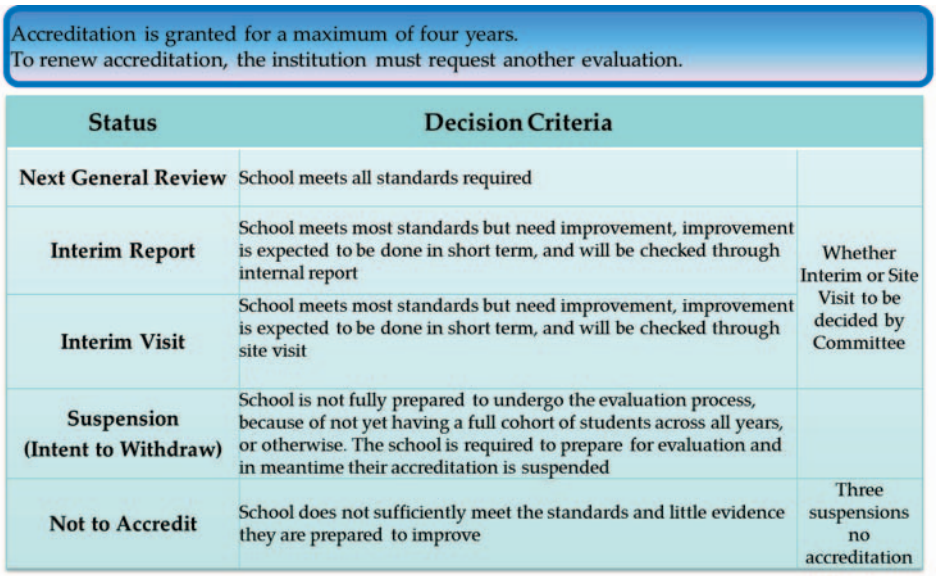

Figure 4. Cycles & status of accreditation

Table 2. Evaluation scheme of the 1st cycle

Year	Number of schools	Name of School
2009	0	
2010	2	· Kyungpook National University, School of Dentistry · Chonnam National University, School of Dentistry
2011	4	· Chosun University, School of Dentistry · Chonbuk National University, School of Dentistry · Gangneung-Wonju National University, College of Dentistry · Kyung Hee University, School of Dentistry
2012	5	· Wonkwang University, School of Dentistry · Yonsei University, College of Dentistry · Dankook University, School of Dentistry · Seoul National University, School of Dentistry · Pusan National University, School of Dentistry

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Undergraduate Dental Education in the United Kingdom: Curriculum Design and Regulation.

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ABSTRACT

In the UK there are 16 dental schools providing undergraduate dental education, graduating about 1120 new dentists per year, for a population of 63 M people. The dental course is 5 years in duration and culminates in the award of a bachelor's degree (BDS), which entitles the holder to register as a dentist with the General Dental Council (GDC).

The GDC is responsible to the UK Government for patient safety and for the quality of the dental training programmes. To exercise this responsibility, the GDC inspects all dental schools on a regular basis. The schools must meet minimum standards for providing dental education and all new graduates must meet 149 learning outcomes before they are considered fit to register and practice dentistry. To assist the school, these standards and learning outcomes are published in detailed documents.

The UK must also meet the directives of the EU, which ensure that all dentists in the 28 European countries have had a similar basic dental training. A dentist who graduates in any member state can then register and practice in any other member state, without restriction.

After graduation UK dentists must undertake one further year of Foundation Training — a period of supervised and mentored practice in primary care under the supervision of an experienced practitioner. All UK graduate dentists must satisfactorily complete this Foundation Year before they are allowed to work for the National Health Service. EU graduate dentists are exempt from this requirement and are allowed to work with no further training or restrictions.

Dental Schools use the guidelines from the GDC to develop and design their undergraduate courses, but also take advice from specialist societies and education groups who publish guidelines regarding the core subjects that should be taught.

INTRODUCTION

In the United Kingdom there are a total of 18 Dental Schools, 16 of which train undergraduate dental students (Table 1). Two Schools are postgraduate only and provide specialist training and postgraduate degrees, mostly at Masters level. Overall therefore there are 16 schools that between them train approximately 1120 new dentists per year to serve a UK population of 63 million.

Dental education in the UK is an undergraduate degree course (Bachelor of Dental Surgery (BDS)). Candidates may enter dental school direct from (high) school at the age of 18 (Year 13) after taking their advanced level (A Level) examinations. There is no national university entrance examination in the UK. School students in year 11 will choose which subjects they will study to A level, taking account of which university or university course they wish to apply for. In the UK most students sit 3 or 4 A level subjects. To gain a place to study Dentistry they will normally be required to sit at least two science subjects at A level and to obtain the top grade (A) in all subjects. Most entrants into dental school will have studied chemistry, biology and physics. For most dental schools there are about 10 applicants for every place.

It is also possible for students to enter dental school with a previous science degree. If this degree covers subjects relevant to the dental course, they may be awarded

Table 1. The eighteen dental schools of the United Kingdom

England
King's College London
Newcastle University
Plymouth University
Queen Mary, University of London
* University College London (Eastman Dental Institute)
University of Birmingham
University of Bristol
University of Central Lancashire
University of Leeds
University of Liverpool
University of Manchester
University of Sheffield
Wales
Cardiff University
Scotland
University of Aberdeen
* University of Edinburgh (Postgraduate Dental Institute)
University of Dundee
University of Glasgow
Northern Ireland
Queen's University Belfast

* These two Schools are postgraduate only

an exemption of one year.

AN OVERVIEW OF UNDERGRADUATE DENTAL EDUCATION IN THE UK

The undergraduate degree programme for dentistry is 5 years in duration and culminates in the award of a Bachelors degree (BDS). Traditionally this five year course has been divided into approximately two years of "basic sciences" and three years of clinical studies. However, almost all schools now provide an integrated curriculum where teaching and learning of basic sciences is integrated with clinical skills from the first semester.

Figure 1 shows a simple model of the Sheffield curriculum. In the very first year students will begin to acquire clinical skills at the same time as learning the basic sciences. While learning about normal structures they will learn about abnormalities or disease, and begin to learn the basics of how to take a clinical history. At the same time they will have courses on communication skills, ethics and consent. For example, while learning about the anatomy of the teeth and periodontal tissues, they will also learn about dental caries and periodontal disease, and will be introduced to dental instruments and will learn how to scale and polish on each other. They will learn how to set up a dental unit and about the principles of disinfection and cross infection control.

The students will see their first patients at the beginning of semester 1 of second year. They will undertake clinical examinations, learn how to take a proper history and take impressions for the construction of dentures. During this semester they also take a basic clinical skills course in the simulation laboratory, using plastic or extracted teeth to learn the skills of restorative dentistry. They must pass this course to be allowed to undertake invasive non-reversible treatments on patients. In semester 2 of second year they begin restorative dental treatment on their own patients. Students then continue clinical treatment of patients with increasing independence until they graduate. By the 5th and final year they will have taken responsibility for the whole care of a number of patients.

Most dental schools in the UK have a similar course, characterised by integration of knowledge and skills and early contact with patients.

Another feature of dental education in the UK is the increasing use of outreach placements, where students go out to work in the "real world" of dentistry. This may be in community clinics or in general dental practices (private dental offices). In these environments they are trained and supervised by primary care dentists in their own practices or clinics. Some schools (Plymouth, Lancaster and Aberdeen) now do almost all of their clinical training in community clinics. The remaining schools do most of their clinical training in their own clinics or dental hospital. In Sheffield the senior students (4th and 5th years) undertake 20 weeks of outreach training in primary care in private dental clinics or community clinics (Figure 1). This is one of the largest outreach programmes in the UK and the only one that trains in private clinics. Other schools provide shorter attachments or send students out on a day-release basis.

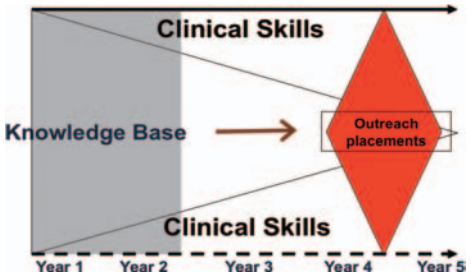

Figure 1. A simple overview of the integrated dental course at Sheffield. Key features include early introduction to clinical skills and a period of outreach in primary care in the 4th and 5th years.

OPPORTUNITIES ON GRADUATION

The undergraduate training of dentists is regulated and quality assured by the General Dental Council (see below) who essentially "accredit" each of the university BDS programmes. On graduation, each student is awarded the BDS degree and can then be automatically registered as a dentist with the General Dental Council. In the UK today there are 40,000 registered dentists, and approximately 2,500 new dentists register each year (GDC, a). It can be seen therefore that less than 50% of newly registered dentists each year graduate from UK dental schools. The remainder enter the UK from other European countries, or may have qualified elsewhere in the world and have sat the GDC "Overseas Registration Examination" (ORE).

Since 1993, all newly registered UK graduates must undertake one year of supervised postgraduate clinical training. This is called Dental Foundation training (sometimes also called Vocational Training) and is undertaken in general dental practices. Young dentists are mentored and supervised by experienced dentists in their own practice. This programme is funded by the UK Departments of Health and is compulsory if a dentist wishes to work in the National Health Service. If a new graduate does not undertake this additional year of supervised training they are only permitted to work in private practice or may enter the hospital service to train as a specialist. Because of anomalies in EU and UK laws, dentists who graduate in other EU countries are exempt from this additional year of training and can work for the National Health Service without restriction.

After Foundation training about 90% of dentists continue to work in primary care. The remainder may do one or more additional years of training in hospitals, and some will then undertake further training to be a specialist.

REGULATION OF DENTAL EDUCATION IN THE UK

The dental profession is regulated overall by the UK government by an Act of Parliament called the Dentists Act 1984 (Dentists Act, 1984). This act sets the rules and laws as to how the profession should be regulated and who can or cannot practice dentistry. The implementation and policing of the act is delegated by the Government to the General Dental Council (GDC, b) who are responsible for regulating the whole of the dental

profession.

A complication is that the UK is part of the European Union, which comprises 28 member states across Europe. The Member states have various agreements relating to free trade, but also to recognition of qualifications and free transfer of the workforce, so that a dentist who is registered in one member state, may automatically register and work in any other member state. This means that any dentist who has qualified in Europe may register with the General Dental Council and work in the UK.

The role of the European Union

The EU issues Directives, which set out the agreements under EU law that all member states must follow. With regards to dental qualifications the UK is bound by "Directive 2005/36/EC of the European Parliament and Council on the Recognition of Professional Qualifications" (European Union). This sets out the requirements of training of many groups of professionals including dentistry, so that member states can have confidence that there is uniformity across the countries. With regards to dentistry, the Directive states that a "Basic dental training shall comprise a total of at least five years of full time theoretical and practical study, comprising at least the programme described in Annex V, Point 5.3.1 and given in a university, in a higher institute providing training recognised as being of an equivalent level or under the supervision of a university." Annex V of the Directive lists the subjects that must be taught (Table 2).

The Role of the General Dental Council (GDC)

The GDC must satisfy the UK Government that all Dental Schools abide by the EU Directive and must also regulate the quality of undergraduate (and postgraduate) education. To do this, the GDC undertake periodic inspections of all the UK schools — normally once every 5 years. The schools are inspected against a set of "Standards for Education" (GDC, c), which sets the "Standards and requirements for providers of education and training programmes". In this document the GDC set out 29 requirements that each School must meet across four domains (Table 3). At each inspection the school must produce documentary evidence that the each of the requirements has been met.

In addition the GDC sets out the learning outcomes that any dental registrant must have achieved before they can be registered to practice. These outcomes are described in the GDC document "Preparing for Practice. Dental team learning outcomes for registration" (GDC, d). The learning outcomes reflect the knowledge skills, attributes and behaviours that are required for a dental care professional to practice safely. To be registered as a dentist, an individual must demonstrate that they have met 149 outcomes across 4 domains. The domains and examples of outcomes are given in Table 4.

To be able to award the dental degree and ensure that students are eligible to register, each school must be able to demonstrate that all students have been taught, and have been assessed on, each of the outcomes. The GDC test this by close scrutiny of each schools curriculum and assessment processes. In practice, each school demonstrates this by mapping their curriculum and assessments against the outcomes.

Curriculum design and benchmarking

In the context of this complex regulatory framework, how do dental schools decide what subject should be taught?

As a starting point UK schools must ensure that their curriculum includes all the topics covered in the EU Directive and the GDC Learning Outcomes. The detail of subjects to be taught is then guided by experience and by custom and practice. However there is clear evidence that the nature of the workforce and the oral health needs of the population are changing and that dental education

Table 2. List of subjects that must be taught in a programme of study leading to a dental qualification. From the EU Directive on professional qualifications (Annex V. 5.3.1) (4)

A. Basic subjects	B. Medico-biological subjects and general medical subjects	C. Subjects directly related to dentistry
Chemistry	Anatomy	Prosthodontics
Physics	Embryology	Dental materials and equipment
Biology	Histology, including cytology	Conservative dentistry
	Physiology	Preventive dentistry
	Biochemistry (or physiological chemistry)	Anaesthetics and sedation
	Pathological anatomy	Special surgery
	General pathology	Special pathology
	Pharmacology	Clinical practice
	Microbiology	Paedodontics
	Hygiene	Orthodontics
	Preventive medicine and epidemiology	Periodontics
	Radiology	Dental radiology
	Physiotherapy	Dental occlusion and function of the jaw
	General surgery	Professional organisation, ethics and legislation
	General medicine, including paediatrics	Social aspects of dental practice
	Oto-rhino-laryngology	
	Dermato-venereology	
	General psychology, psychopathology, neuropathology	
	Anaesthetics	

Table 3. GDC Standards for Education. The four domains of the standards and the 29 requirements that each School must meet.

Standard 1 Protecting Patients	Standard 2 Quality Evaluation & Review of the Programme	Standard 3 Student Assessment	Standard 4 Equality & Diversity
1. Students must provide patient care only when they have demonstrated adequate knowledge and skills. For clinical procedures, the student should be assessed as competent in the relevant skills at the levels required in the pre-clinical environments prior to treating patients	9. The provider must have a framework in place that details how it manages the quality of the programme which includes making appropriate changes to ensure the curriculum continues to map across to the latest GDC learning outcomes and adapts to changing legislation and external guidance. There must be a clear statement about where responsibility lies for this function	16. To award the qualification, providers must be assured that students have demonstrated attainment across the full range of learning outcomes, at a level sufficient to indicate they are safe to begin practice. This assurance should be underpinned by a coherent approach to aggregation and triangulation, as well as the principles of assessment referred to in these standards.	27. Providers must adhere to current legislation and best practice guidance relating to equality and diversity
2. Patients must be made aware that they are being treated by students and give consent	10. The provider must have systems in place to quality assure placements	17. The provider will have in place management systems to plan, monitor and record the assessment of students throughout the programme against each of the learning outcomes.	28. Staff must receive training on equality and diversity, development and appraisal mechanisms will include this
3. Students will only provide patient care in an environment which is safe and appropriate. The provider must comply with relevant legislation and requirements regarding patient care.	11. Any problems identified through the operation of the quality management framework must be addressed as soon as possible	18. Assessment must involve a range of methods appropriate to the learning outcomes and these should be in line with current practice and routinely monitored, quality assured and developed.	29. Providers must convey to students the importance of compliance with equality and diversity law and principles of the four UK nations both during training and after they begin practice
4. When providing patient care and services, students are to be supervised appropriately according to the activity and the student's stage of development.	12. Should quality evaluation of the programme identify any serious threats to the students achieving learning outcomes through the programme, the GDC must be notified immediately. (n.b. where there is geographical variation in oral health needs, providers must inform the GDC of the issues and action to be taken to demonstrate that the outcomes have been met	19. Students must have exposure to an appropriate breadth of patients/procedures and should undertake each activity relating to patient care on sufficient occasions to enable them to develop the skills and the level of competency to achieve the relevant GDC learning outcomes	
5. Supervisors must be appropriately qualified and trained. Clinical supervisors must have appropriate general or specialist registration with a regulatory body.	13. Programmes must be subject to rigorous internal and external quality assurance procedures	20. The provider should seek to improve student performance by encouraging reflection and by providing feedback.	
6. Students and those involved in the delivery of education and training must be encouraged to raise concerns if they identify any risks to patient safety.	14. External examiners must be utilised and must be familiar with the learning outcomes and their context. Providers should follow QAA guidelines on external examining where applicable	21. Examiners/assessors must have appropriate skills, experience and training to undertake the task of assessment, including appropriate general or specialist registration with a regulatory body	
7. Should a patient safety issue arise, appropriate action must be taken by the provider.	15. Providers must consider and, where appropriate, act upon all concerns raised, or formal reports on the quality of education and assessment	22. Providers must ask external examiners to report on the extent to which assessment processes are rigorous, set at the correct standard, ensure equity of treatment for students and have been fairly conducted.	
8. Providers must have a student fitness to practise policy and apply as required. The content and significance of the student fitness to practise procedures must be conveyed to students and aligned to GDC student fitness to practise guidance. Staff involved in the delivery of the programme should be familiar with the GDC Student Fitness to Practise Guidance.		23. Assessment must be fair and undertaken against clear criteria. Standard setting must be employed for summative assessments.	
		24. Where appropriate patient/peer/customer feedback should contribute to the assessment process	
		25. Where possible, multiple samples of performance must be taken to ensure the validity and reliability of the assessment conclusion.	
		26. The standard expected of students in each area to be assessed must be clear and students and staff involved in assessment must be aware of this standard.	

Table 4. GDC Learning Outcomes. The four domains and examples of learning outcomes in each domain. In total there are 149 learning outcomes.

Domains			
Clinical	**Communication**	**Professionalism**	**Management & Leadership**
Identify oral diseases and explain their relevance to prevention, diagnosis and treatment.	Recognbise the imprtance of non-verbal communication. Including listening skill, and barriers to effective communication.	Put patients interest first and act to protect them.	Effectively manage their own time and resources.
Recognise the responsibilities of a dentists as an access point to and from wider healthcare.	Obtain informed consent.	Act without discrimination and show respect for patients, colleagues and peers and the general public.	Recognise the importance of and demonstrate personal accountability to patients, the regulator, the team and wider community.
Assess, diagnose and manage the health of the dental pulp and periradicular tissues, including treatment to prevent pulpal and periradicular disease.	Use appropriate methods to provide accurate, clear and comprehensive information when referring patients to other dental and healthcare professionals.	Recognise and evaluate the impact of new techniques and technologies in clinical practice.	Decribe the legal, financial and ethical issues associated with managing a dental practice.

must evolve to accommodate these changes (Wilson et al., 2008). Across Europe the Association for Dental Education in Europe acts as a forum for dental educators and convenes regular working groups to develop consensus guidelines for the design and development of the undergraduate curriculum (Plasschaert et al., 2006. Plasschaert et al., 2007) and have prepared a detailed list of competences that have informed schools and the GDC (Cowpe et al., 2010). The specialist societies also issue guidelines on the ideal content of a dental curriculum and the core subjects that trainee dentists must know. For example, specialist working groups have issued guidelines on curriculum content for Oral Surgery (Macluskey et al., 2008), Oral Pathology (Odell et al., 2004) and Cariology (Anderson et al. 2011). Using these guidelines and through regular meetings of education interest groups and specialist societies, the UK schools are able to benchmark their curricula against each other and ensure compliance with the regulators. In the UK, there is also an overarching council of all the heads of dental schools (Dental Schools Council) -, which meets regularly and convenes working groups as appropriate.

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Dental Curriculum, Accreditation and Licensure: A North American perspective

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INTRODUCTION

In North America there are critical links between dental curriculum, dental accreditation and dental licensure. Importantly North American dental graduates can enter practice in the year after graduation so they must be prepared to be licensed in order to practice dentistry and address the oral health needs of their patients. In this paper I will review the history of dental education and the ways that current curricula reflect the historical perspective. As the dental profession developed the necessity of regulatory bodies to protect the patient and insure that the dentists in practice were qualified were organized. Over time this regulation resulted in the development of testing methods to determine if a newly registered dentists fulfilled the criteria established to define a qualified dentist. These standards became linked to dental curricula to insure that graduates were appropriately prepared. Dental accreditation developed as an external metric to normalize dental education to an accepted standard and insure that graduates of all the dental schools were adequately prepared to enter practice. In this paper I will present an overview of the history that resulted in the current structure of North American dental education and how this was incorporated in accredited programs of dental education that prepared the graduates to meet the qualifications for dental licensure.

HISTORY OF DENTAL EDUCATION IN NORTH AMERICA

Patients received dental care from many different providers in the 1800's, including barber-surgeons, blacksmiths, and carpenters. At this time much of the treatment involved the extraction of teeth and individuals skilled with tools were viewed as a potential dental provider. The history of dentistry in North America is very well presented in an online resource, *Dental History*, that was developed by the American College of Dentists[1]. Some highlights from that publication that have particular relevance to the topic of this paper and the presentation in the meeting follow. The first formal dental school in North America was developed in Baltimore in 1840, the Baltimore College of Dental Surgery, which incorporated a science-based curriculum in the 2 partial years of study. That school continues to be represented in North American dental education as the School of Dentistry at the University of Maryland. The Philadelphia Dental College opened in 1863 and was eventually incorporated into Temple University. The first University-based dental school opened at Harvard University in 1867. In Canada the Royal College of Dental Surgeons opened in Toronto in 1870 and was eventually incorporated into the University of Toronto. McGill and the University of Montreal opened dental schools in 1905. As dental education programs became incorporated in universities the rigor of the curriculum increased with ever more scientific content incorporated to help explain the etiology and pathogenesis of oral diseases and the basis for approaches to treatment. However in the first 25 years of the 20th Century dental education in North America was a very uneven process with many dentists learning through apprenticeships and in proprietary dental schools. Medical education in the United States and Canada had had a similar variety of education approaches and in 1910 Dr. Abraham Flexner lead an analysis examining medical education in the United States and Canada[2]. Based on this report medical education made a dramatic change and leaders in dental education realized that a similar analysis of dental education was required. The links of science and human disease were made and began to be incorporated in health science education.

The modern era of dental education in North America can be directly traced to the publication of the Carnegie Report on Dental Education in the United States and Canada by Dr. William J Gies[3]. Often referred to as "The Gies Report," this document from 1926 clearly presented the variety of ways that dentists were educated/trained and identified deficiencies in some of the educational approaches and the educational elements necessary for dentistry to be viewed as a profession. Prior to 1926 dental education occurred in multiple formats including apprenticeships, proprietary schools and some university-based dental schools. The Gies Report fundamentally changed the way that dental education was provided and eventually lead to changes that moved all dental education into a University setting. The change to a university setting also changed the curriculum, increasing the quantity of basic science education included and reinforcing the importance of research as an essential activity in a dental school. The result was that most dental schools eventually became 4 years in duration with expanded content in the basic biomedical sciences. The increased basic science content also affected the requirements for admission to dental schools and in the next 25 years an increase in the amount of prerequisite undergraduate university science courses occurred that ultimately lead to the situation today where nearly all students admitted to North American dental schools

have a 4-year Bachelor's degree. The linkage of dental education with Universities enhanced the rigor of the dental curricula and ultimately normalized the dental education for students attending these programs.

DENTAL ACCREDITATION IN NORTH AMERICA

The move of dental education programs to Universities lead to a drive to develop criteria to insure that all programs graduated a similarly prepared new dentist. The evolution of dental accreditation during the last half of the 20th century has resulted in the current sets of guidelines and processes. In the United States the American Dental Association Council on Dental Accreditation (CODA) is the official accrediting body for all US dental schools. In Canada the Commission on Dental Accreditation of Canada (CDA) has a similar role for the ten Canadian dental schools. Importantly accreditation is not an "examination-type" review but rather an assessment of whether a dental school is achieving its stated goals for dental education. CODA and CDAC play important roles in insuring that the dental education programs meet the objective to prepare a new dentist to enter private practice.

The CODA accreditation process occurs every 7 years for US dental schools. Each school completes a self-study of their curriculum, usually involving a 2 year process. The Self-Study is organized to respond to the standards that have been established by CODA. There are 6 Standards; 1) Institutional Effectiveness; 2) Educational Program; 3) Faculty and Staff; 4) Educational Support Services; 5) Patient Care Services and 6) Research Program[4]. Each Standard has a set of criteria that have been developed to define the characteristics of the curriculum in order that a competent "new beginner" dentist graduates. However each dental school determines the method to be used to achieve the curricular objectives. The Self-Study is reviewed by an accrediting group that consists of multiple stakeholders in dental education and the dental profession and includes faculty members from other accredited dental schools. The accreditation team conducts a site visit at the dental school to review the elements of the self-study and obtain answers to questions that the review of the Self-Study may have generated. Based on the site visit and the review of the Self-Study the site visit team will develop a set of suggestions that could be implemented to improve the dental education program and a set of recommendations that must be implemented to address deficiencies that have been identified. The site visit team makes a recommendation to the full American Dental Association Council on Dental Accreditation, which ultimately makes the decision concerning the accreditation status of the dental education program. For programs that are fully operational the two accreditation outcomes exist, which are either Approval (without reporting requirements) or Approval (with reporting requirements). Schools that received recommendations during the accreditation site visit must report to CODA on progress to address those deficiencies. Graduates of accredited US dental programs have reciprocity in Canada as due graduates of accredited Canadian programs in the US allowing graduates of dental schools in either country to progress through the steps required for licensure in either country. The external reviewers on the site visit team provide an excellent perspective on a dental curriculum and can provide important insights to improve a program.

The Commission on Dental Accreditation of Canada functions in a manner very similar to CODA. Dental education programs are reviewed on a 7 year cycle, a set of 7 standards have been developed including: 1) Institutional Structure; 2) Educational Program; 3) Administration, Faculty and Faculty Development; 4) Education Support and Services; 5) Clinic Administration; 6) Research and Scholarly Activities and 7) Program Relationships[5]. Each standard has several subdivisions that establish the criteria that need to be included in the Self-Study of the dental curriculum. An accreditation site visit occurs with a team of faculty members from other dental schools knowledgeable on the topics to be reviewed. The site visit team generates a report with Suggestions and Recommendations based on their review and submit that report to the CDAC Board for a final decision. The final decisions for an operational program are either Approval without reporting requirements or Approval with reporting requirements. The reports required provide evidence that the deficiencies noted in the Recommendations are being addressed. The similarity between the CODA and CDAC processes is important as the United States and Canada have reciprocity for dental degrees earned in either county. In addition Canada has established reciprocity agreements with accredited dental education programs in Australia, Ireland and New Zealand and in the province of Quebec there is reciprocity with dental education programs in France. The reciprocity agreements mean that dental students graduating from these dental education programs in these countries are eligible to sit the National Dental Examination Board in Canada and if they pass the exam and have appropriate immigration status they are eligible to apply for dental licensure in any Canadian province. The reciprocity of Canada with the other countries does not extend to the United States. The similarity of the CODA and CDAC standards and processes means that dental education in Canada and the United States is very similar.

CURRICULUM STRUCTURE AND CONTENT

North American dental curricula are all based on a 4 year program of study. Entering students typically have completed a 4 year baccalaureate degree prior to admission to provide the necessary background to successfully achieve the biomedical science learning objectives. In both the US and Canada dental admissions are quite competitive and admission is based on undergraduate grade point average, performance on a Dental Admissions Test (DAT) and completion of a school-specific interview process. Each school does dental admissions individually and a student may apply to a very large number of schools and go through the entire process multiple times. Analysis of the criteria for admission and success in the dental curriculum has shown that GPA and the basic science elements of the DAT are positively correlated with student success with mastery of the didactic content of the curriculum. The

Perceptual Motor Ability (PMAT) component of the DAT has been shown to be positively correlated with student achievement in pre-clinical simulation learning although the correlation is not as strong[6]. Many other types of assessments of psychomotor ability have been attempted but none of them have been shown to have a predictive ability for students learning the technical aspects of dentistry. In the past decade the numbers of applicants to dental school has increased and the academic preparation of the students accepted has improved.

In examining a North American dental curriculum there are three major elements; 1) a didactic element that covers the basic biomedical sciences, the behavioral sciences and the clinical sciences; 2) the pre-clinical simulation experiences with dental procedures; and 3) clinical patient care. There is clearly a progression of this content in developing the competency goals for a new graduate however the way that the content is organized in each dental curriculum is dependent on the plans of faculty members at the specific institution. While there are some similarities between dental schools it is likely that no two dental schools have a curriculum that is identically organized. Three generic structures for dental curricula have been depicted in the IOM Report (p.96)[7]. In a traditionally organized dental curriculum, which likely represents the majority of dental schools, the first year is primarily biomedical science courses, the second year clinical science and pre-clinical simulation and the third and fourth years dedicated to patient care. The variations on this typical organization incorporate clinical science/activity earlier and continue to emphasize biomedical science principles in the later years. All of the curricular structures have been assessed in accreditation processes and no school has ever been criticized specifically for the way the content is distributed over the four years of the dental curriculum. In addition to differences in the structure of the four years there are also differences in the linkages to health science education in other disciplines like medicine. In some schools the medical and dental students participate in the same learning experiences in the first two years and when medical students move on to their clinical clerkships the dental students enter the dental clinic with direct patient care. Another difference is in pedagogy used by different schools. The predominant pedagogy in North American dental schools is very traditional with classic lecture presentations by faculty experts and assessment of the student with factual recall type examinations. The schools that have medical and dental students learning together also tend to have fact-recall types of student assessments. It is unusual to have student assessments of critical thinking ability or that require integration of knowledge to address a problem. Some schools have used problem based learning (PBL) pedagogy to develop critical thinking skills and integration of knowledge. These schools have also incorporated student assessment methods that are not simply factoid recall but require critical thinking and application of multiple learning topics[8]. There is an increasing emphasis on interprofessional education (IPE) in North American dental education and it is likely that more students in a variety of health education curricula will learn with, from and about other health professional students. Since all the health professions are treating the same species there is considerable overlap that occurs in the basic science content knowledge that is included in each of the different professional curricula and it could be imagined that a common experience in learning could ultimately benefit patient care by the different professionals working together. It is also likely that all the health professional curricula should be building the same set of professional values in their students, which would make shared ethics/professionalism learning a benefit. The greatest variation in the different health professional curricula is linked to the specific skills required for each profession, in this regard dentistry requires the development of a considerable skill set required to provide the highest quality oral health care to patients. Accreditation normalizes the various dental curricular so that each dental graduate should have the necessary knowledge, skills and values to provide the highest quality oral health care.

In the nearly 90 years since the Gies Report, dental curricula have evolved. To assess the status of that evolution and the current state of dental education the Institute of Medicine of the National Academy of Sciences of the United States organized a panel to review dental education. That panel published the report, *Dental Education at the Crossroads*, in 1995[7]. The IOM Report was critical of several aspects of the existing dental education including; weak links between basic and clinical sciences, overcrowded curriculum, weak linkages between dentistry and medicine, difficulty with comprehensive patient care, and too many dental schools and dental faculty members minimally involved in research and scholarship[7]. Interestingly many of the concerns identified by the IOM Panel were similar to points made by Gies in 1926[3]. In particular Gies was a very strong proponent of an increased research profile in dental schools and by dental faculty members. The IOM Report had 22 Recommendations for changes in dental education[7]. These recommendations pertained to the full breadth of the teaching, research and service missions of a dental school. Implementation of all these recommendations would have been a major undertaking for any one dental school and likely would have had a considerable financial impact. The IOM report catalyzed some active introspection of dental curricula and one aspect that came about were some pedagogical changes structured to address some of the concerns contained in the IOM Report. The IOM Report also generated considerable controversy and in some cases defensiveness about the state of dental education in 1995. Many dental schools and faculty members recognized the concerns raised by the IOM but few dental schools and dental curricula made significant changes to address the concerns. Some incremental progress was made and reported by individual faculty members in presentations at the ADEA Annual Meeting and in the Journal of Dental Education. The faculty members working on those topics were eventually organized into the ADEA Commission on Change and Innovation in Dental Education (CCI). The CCI cohort began a focused set of meetings to exchange best practices and publish the results of their examinations of change and innovation in dental curricula. An impressive set of 22 publications resulted from the work of the

CCI and all these publications were incorporated in a new book called, Beyond the Crossroads[9]. The CCI group has become an important venue for faculty members to share their educational practices and begin to identify innovations that can address the concerns raised by the IOM Report. The work of the CCI group continues and additional publications have resulted, which provide important information to dental faculty members at other dental schools on approaches to improve the effectiveness of a dental curriculum.

The requirements to graduate from dental school have also evolved. One important aspect with respect to graduation from either a US or Canadian dental school is that the new graduates are able to enter private practice immediately after passing the necessary licensing examinations/procedures. Therefore dental schools have established the goal that a new dental school graduate is a "safe beginner." For many years this was interpreted that a new graduate had had sufficient clinical experiences as a dental student to have the clinical skills required to treat dental patients independently. Thus there were fixed numerical requirements for specific procedures that represented the standard for a dental graduate. These requirements were a major focus for the dental students and in some cases the mindset was that "teeth" were treated rather than "patients." The fixed procedural numbers also emphasized "procedures" at the expense of application and reinforcement of the role of the biomedical sciences in patient care. As the variety of approaches to patient care increased in number the breadth of clinical skills expanded and fixed numbers became a barrier to the proper care of patients. The IOM Report pointed out some of these weaknesses in dental curricula and beginning in the 1990's the goal of dental education moved to a competency-based approach[7]. Graduation competencies have gone through several iterations and currently the ADEA Competencies have changed from more than 45 individual competencies to 6 domains of competency including; 1) Critical Thinking; 2) Professionalism; 3) Communication and Interprofessional Skills; 4) Health Promotion; 5) Practice Management and Informatics and 6) Patient Care, which has 2 sub-domains; A) Assessment, Diagnosis and Treatment Planning and B) Establishiment and Maintenance of Oral Health[10]. Each of the Competencies has different categories that help to define the domain level competency. The ADEA competencies are recommendations not requirements for each US dental school and any school could have a school-specific set of competencies. The ACFD in Canada has defined 47 competencies for the new dentist, these are very similar to the original ADEA competency list that existed prior to the grouping of the individual competencies into domains[11]. ACFD is currently reviewing the competencies with the intent to group them into categories that are more global and similar to the manner that ADEA has listed the competencies. Determining that an individual student has achieved competency remains an important objective. Each school measures the achievement of competency in a school-specific manner that is generally linked to successful completion of courses and patient care. The topic of measuring competency is one that is discussed at many meetings of ADEA and ACFD. Competency may be defined as the mastery of a core body of knowledge, a sufficient breadth of clinical patient care experience, specific clinical performance assessments of a student treating a patient independently and the clinical decision-making abilities to decide what approach to care is appropriate for a given patient, with a given diagnosis and the desired patient specific outcomes. The mechanism to assess each individual student in all four of these competency components remains in development and considerable new research on these assessments is required. The importance of measuring competency is enhanced since both CODA and CDAC accreditation processes have both moved to assessment of competency-based dental curricula, which has resulted in far less reliance on specific numbers of procedures as a metric for graduation. Competency based education is used in most of the health professions and it is likely that in the future the process of assessing competency will have increased measures of reliability and validity that will be based on educational research outcomes.

DENTAL LICENSURE IN NORTH AMERICA

Dental licensure and regulation of the dental profession occurs at the level of the state in the United States and the province in Canada. The result is that each state may have a slightly different procedure to license a dentist and each dentist must be licensed by the appropriate state or provincial regulatory body in order to treat patients in that state/province. In the United States there are 5 components that are common to licensure in every state; 1) graduation from an accredited dental school in either the United States or Canada, 2) the candidate must pass Part 1 of the National Board Dental Eam (NBDE) an exam of the basic science content in the dental curriculum; 3) must pass Part II of the NBDE, which is an assessment of the clinical science content of the dental curriculum: 4) pass a patient-based examination on specific patient treatments, there are several different regional boards that administer these exams and some states administer a state-specific board exam, each state regulatory board selects the patient-based exam that will be accepted for licensure; 5) a jurisprudent exam that assesses the candidates knowledge of the laws that regulate the profession in that state. A candidate must pass all 5 elements in order to receive a license. Once licensed most states have a continuing education requirement to demonstrate that dentists remain current with the profession. Canadian licensure is also regulated by the provincial Colleges of Dental Surgeons such that dentistry is a truly self-regulating profession in Canada. The regulatory colleges require that each applicant for licensure graduate from an accredited dental school recognized by CDAC and successfully pass the examination administered by the National Dental Examining Board of Canada (NDEB). Thus in Canada successful graduation from a dental school and successful completion of the NDEB results in licensure, the only variation on that theme is in the province of Quebec where an applicant must also successfully complete an examination of proficiency in French language. The ability of a North American graduate to immediately enter practice following graduation and successful challenge of the necessary licensing exam-

inations has a major impact on dental curricula since they must prepare the new graduate to pass the exams successfully. In the US a dental license is not portable to another state and a candidate must have completed all the requirements of the new state, which may mean that moving to a new state requires the individual to take a different patient-based board exam. In Canada if a dentist has been registered in one Province as a dentist they are eligible to apply for licensure in another province without another exam however the French language exam is required for a dentist who wants to move to Quebec and practice. The requirements for licensure do have impact on curriculum design and can in some ways force dental schools to create curricula that prepare dental students to pass the examinations. If the examinations are primarily recall then the students may not perfect the critical-thinking and problem-solving skills that are essential to excellent practitioner.

CONCLUSION

Dental curriculum, dental accreditation and dental licensure have an intimate relationship. Ultimately the dental graduate will need to be licensed in order to practice. The research achievements in the health sciences are occurring at an incredible rate. It is important that dental graduates leave school at the cutting edge of the science. However modifications of curriculum to reflect the current scientific understanding may conflict with the requirements of accreditation and dental licensure, which are likely not evolving at the same pace as the science. A continuing challenge for all dental educators is to prepare our graduates for the future, which will be much different than the dental profession that exists at the time of dental education. Building the capacities for critical-thinking and problem-solving will be essential to create life-long learners who will continue to incorporate the latest advances in their treatment of patients. Dental curricula need to build skill sets in their students that are applicable throughout their careers rather than simply transfer content knowledge that will likely be outdated in a few short years. Dynamic changes in dental curricula should become the norm and static dental curricula should be avoided.

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Accreditation system for pharmaceutical education in Japan

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Key words: accreditation, pharmacy degree, peer review, third-party evaluation, 6-year pharmacy program, model core curriculum

ABSTRACT

As professionals, needs of the society towards pharmacists have been growing. In response to the social needs, the six-year pharmaceutical education system, which needs for a mandatory registration examination to become a licensed pharmacist, was established by the "Pharmacists Law" and the "Fundamentals of Education Act" in the academic year 2006. In accordance with the amendment of the "Fundamentals of Education Act", it was obligatory us to construct an accreditation system for the 6-year pharmaceutical education in Japan. Then the committee for accreditation system for pharmaceutical education in Japan was set up under the committee of pharmacy education reform in Pharmaceutical Society of Japan to investigate the accreditation system and to draw up a draft of the evaluation standards. The draft was distributed in the end of January 2007 to ask feedback from each pharmaceutical university and had been brushed up through several trails, then established as "The Evaluation Standards 2011 for Pharmaceutical Education" in October 2011. The Japan Accreditation Board for Pharmaceutical Education (JABPE) was set up in December 2008 in order to do the third-party evaluation of pharmaceutical education. The first three universities have been taking the full-scale examination by JABPE in 2012-2013 and all school/college of pharmacy in Japan must take the evaluation by JABPE once in seven years.

INTRODUCTION

In Japan, in response to the social needs, the six-year pharmaceutical education system, which needs for a mandatory registration examination to become a licensed pharmacist, was established in the academic year 2006. With the commencement of 6-year pharmacy education system, the Central Council for Education requested the construction of a system for third-party evaluation of pharmacy education programs, such as Accreditation Council for Pharmacy Education in USA. In order to respond to the request, examination had been carried out mainly by the Pharmaceutical Education Review Committee, established under the University Faculty Conference for the Pharmaceutical Education Reform in the Pharmaceutical Society of Japan and by the Pharmaceutical Evaluation Committee of the Association of Presidents & Deans of Japanese School of Pharmacy. On December 10, 2008 Japan Accreditation Board for Pharmaceutical Education ("JABPE") was formally inaugurated as a general incorporated association. JABPE conducted a full-scale evaluation from March 2012, when the 6-year pharmacy education system was completed.

In the proposal of the Central Council for Education, titled "Toward the Construction of a Bachelor Course Education" (October 2008), field-specific evaluation by a third-party is mentioned as an important issue in the future and universities, colleges, and associated organizations are expected to build a system to guarantee the quality of education according to each field. Under such circumstances, as far as faculty basis in concerned, JABPE is the first organization established in Japan as a voluntary effort to maintain and improve the quality of field-specific education.

NEW EDUCATION SYSTEM IN JAPAN

Table 1 shows the history toward 6-year pharmacy program. The most important epoch making event was establishment of the Model Core Curriculum for pharmaceutical education by the Pharmaceutical Society of Japan in 2002. Based on the Model Core Curriculum finally Committee in the Ministry of Education, Culture, Sports, Science and Technology and Committee in the Ministry of Health, Labor and Welfare determined to extend the educational term of pharmacy program from 4-year to 6-year, and then 6-year pharmacy program was approved in 2004. In 2006 the new 6-year pharmacy program was started in Japan.

The model core curriculum is consist of 81 course credits and includes 1442 specific objects. The core would be 70% of total classes of each university and another 30% is original curriculum, which shows the originality of own university. The model core curricu-

Table 1. History toward 6 -Year Pharmacy Program

1980	A proposal of 6-year pharmacy education by the Japan Pharmaceutical Association
1994	A report of Committee in the Ministry of Health, Labor and Welfare (Extended education for license)
1996	A report of Committee in the Ministry of Education, Culture, Sports, Science and Technology (Extended clinical clerkship)!
2002	The Model Core Curriculum for pharmaceutical education by the Pharmaceutical Society of Japan. Committee in the Ministry of Education, Culture, Sports, Science and Technology was reorganized. Committee in the Ministry of Health, Labor and Welfare was reorganized.
2004	Approval of 6-year pharmacy program!

Table 2. Model Core Curriculum for Pharmaceutical Education

Overview:

- To provide an essential educational content (core content) that every pharmaceutical student are responsible for in various fields in medicine and health care of the 21st century must acquire.
- To suggest the essential importance of implementing an elective curriculum that allows selections by student. (The core would be 70% of total classes)
- To be arranged in 67 units and Practical On-site Training and Graduation Theses research Training Curriculum in 14 units (course credits)
- Specific objectives, given in 1,442 items, are grouped into three areas: knowledge, skill and attitude.
- Pre-pharmacy training designed to integrate classroom knowledge and knowledge gleaned from students' work experience for one month.
- Pharmacy practice is in a community pharmacy setting and a hospital pharmacy setting. Pharmacy practice experience is 5 months in duration and students are expected to be in the practice setting 40 hours per week.

Components of the Model Core Curriculum:

A: humanities & communications
B: introduction to pharmacy
C: professional subjects
  C-1: physics pharmacy
  C-2: chemistry pharmacy
  C-3: biology pharmacy
  C-4: health and environment
  C-5: disease and medicine
  C-6: formulation/manufacturing
  C-7: social pharmacy
D: pharmacy practice
  D-1: preparatory education (in school)
  D-2: practical on-site training (in a hospital pharmacy and a community pharmacy)
E: graduation theses research training
F: general instructional objectives (including liberal arts)
G: advanced pharmacy education

Table 3. History up to the foundation of JABPE

Dec. 2004	Started examination for implementing third-party evaluation as field-specific evaluation
Aug. 2006	The committee of the Pharmaceutical Society of Japan, which includes persons of experience or academic standing formulated and presented as draft of evaluation criteria.
March 2007	Held a briefing session to explain the draft of evaluation standards at two locations in cooperation with the committee of the Association of Presidents & Deans of Japanese Schools of Pharmacy.
April 2007	Conducted a questionnaire survey to colleges about the draft of evaluation standards.
Dec. 2007	Presented the third party evaluation criteria and foundation of JABPE was decided. A foundation preparatory committee was formed.
April 2008	Formed a preparatory committee for foundation of JABPE.
Aug. 2008	Held the second workshop concerning the third-party evaluation of pharmaceutical education.
Dec. 2008	General Incorporated Association, Japan Accreditation Board for Pharmaceutical Education, was founded.

lum also includes total 6 months pharmacy practice. An overview of the model core curriculum is shown in Table 2.

OUTLINE OF JABPE

The history up to the foundation of JABPE is shown in Table 3.

The objective of the incorporated association "JABPE" is to contribute to medical care, public health, and welfare for Japanese citizens by conducting the fair and proper evaluation of pharmaceutical education programs. For this purpose JABPE would guarantee the quality of pharmaceutical education provided by schools/colleges of pharmacy in Japan and thereby enriching and improving education and research activities.

Table 4. Components of the Evaluation Standards 2011

Mission & Goals
1. Mission & Goals

The Curriculum
2. Organization for curriculum
3. Basic Contents of Medical Person Education
   3-1 Humanism Education/Medical Ethic Education
   3-2 Liberal Arts/Language Education
   3-3 Preparatory Education
   3-4 Medical Safety Education
   3-5 Lifelong Learning
4. Pharmaceutical Education Curriculum
   4-1 Model Core Curriculum for Pharmaceutical Education
   4-2 Contents of University Original Pharmacy Professional Training
5. Clinical Clerkship
   5-1 Pharmacy Practice
   5-2 Common Achievement Tests
   5-3 Clinical Clerkship
6. Education for Breeding of Ability for Problem Solving
   6-1 Graduation Research
   6-2 Self-study/Participation Type Learning

Students
7. Admission Policy and System for receiving
8. Scholastic Evaluation/Completion Authorization/Graduation
   8-1 Scholastic Evaluation
   8-2 Completion Authorization
   8-3 Graduation
9. Student Services
   9-1 Study Support System
   9-2 Consideration to Security and Relief

Teacher Organization/Staff Organization
10. Teacher Organization/Staff Organization
   10-1 The Teacher Organization and Faculty Development
   10-2 Education/Research Activities
   10-3 The Staff Organization and Staff Development

Institutions/Facilities
11. Institutions/ Facilities
   11-1 Facilities in the University

Collaborative Relationships
12. Collaborative Relationships with Society

Check & Evaluation
13. Self-check/Self-evaluation

JABPE will perform the following project across the country in order to achieve the objective set forth in the preceding article.

- Evaluation of pharmaceutical education programs.
- Education for the enrichment and improvement of pharmaceutical education programs.
- Research and study concerning the enrichment and improvement of pharmaceutical education programs.
- Publication of journals, academic books, etc. concerning pharmaceutical education programs.
- Information exchange and cooperation with various related organizations.
- Other projects necessary to achieve the objective of JABPE.

In order to establish a system for evaluation of pharmaceutical education programs, JABPE carried out the trail in cooperation with several universities from 2010 to 2011. After improvement of the system, full-scale evaluation in the third-party evaluation of pharmaceutical education about three universities has been done in 2012-2013.

THE EVALUATION STANDARDS 2011

Evaluation of pharmaceutical education is achieved based on The Evaluation Standard for pharmaceutical education program. As shown in Table 3, JABPE made the draft of evaluation standards and had brushed up several times, and then the "Evaluation Standards 2011" was fixed for first seven year's evaluation period. Components of the "Evaluation Standards 2011" are shown in Table 4.

HOW TO PEER REVIEW

Evaluation of pharmaceutical education programs of each university by JABPE is carried out basically by peer review system. The peer review system consists of three steps. First step of reviewing is checking of self-check evaluation book and site visit by a peer review team, which consists of three university teachers and two pharmacists. Next, the report from the peer review team is examined by the assessment committee, which consists of university teachers, pharmacists, medical doctors, nurses, and lawyers, after this examination the assessment committee make the own report. Final step is examination of the report from the assessment committee by the superior assessment committee. The superior assessment committee consists of university teachers, pharmacists, doctors, nurses, and lawyers, journalists, social workers, citizens. In order to make the opinion from society reflect and to correspond to progress in medicine, the peer review team, the assessment committee, and the superior assessment committee consist of respectively different members.

In 2013 three universities is taking examination by JABPE and the first assessment result will be released from JABPE in March 2014. All 73 school/college of pharmacy in Japan have to receive the accreditation of pharmaceutical education by JABPE from 2012 to 2019.

An Introduction of Comprehensive Model Practice Course at Faculty of Dentistry, Niigata University, Japan

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Key words: dental education, model practice, comprehensive, pre-clinical training, treatment planning, simulation

ABSTRACT

We are currently facing a big problem that is society aging. This causes us a shortage of patients for undergraduate student clinic because many patients who are visiting our dental hospital are elderly with complicated oral and total body situation and are not suitable for the student. Therefore, we now have to think how we could have the students effectively learn clinical treatments with limited number of patients. For this purpose, we developed some new practice models that require comprehensive treatment planning practice.

We have developed six different comprehensive models. Each model has teeth that should be extracted, dental caries, missing teeth, accumulation of dental calculus, a multi-root tooth with deep periodontal pocket with the indication of hemi-section or tri-section and a tooth requires endodontic treatment. Some of them have tooth dislocation. Here, we introduce this novel comprehensive model practice course.

We have been experiencing this new program for eight years and the students' responses have been quite positive so far according to the questionnaire performed at the end of this course each year. The assessment is not well established so far but we are trying to use objective methodology with rubric to assess their performance appropriately.

Development of clinical training program for sophisticated dental education

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Key words: inter-university collaboration; undergraduate clinical education; clinical competency; training program

ABSTRACT

The primary mission of dental school is to educate dental students with high competency and to deliver high quality of dental service to the public. In 2006, the common achievement test (CAT) was subjected to all dental students in Japan before starting general practice sessions to credit their competency. However, it has also pointed out that general practice sessions decreased its quality, suggesting the necessity for an immediate improvement of undergraduate clinical training system. Under the MEXT-supported inter-university program, we are developing the sophistication of clinical training of undergraduate dental training by introducing the patient robot system in the advanced skills lab and the manikin-based clinical competency examination (CCE). Our goal for this project is to educate dental students with high competency and to deliver high quality of dental service to the public, by developing the sophisticated dental clinical training program.

BACKGROUND

The dental examination system in Japan: improvements and the remaining issue

In Japan, each dental school provides a 6-year integrated education course consisted of liberal arts and professional education. After graduation of dental school with the degree of Doctor of Dental Surgery (D.D.S), all students are subjected to pass the national exam for dentists (NED) to obtain a dental license. Japanese national dentist exam was started in 1947, and has been administered by the Ministry of Health, Labor and Welfare. The original NED contained both academic and practical examinations to test candidate's knowledge and skills related to dental treatment. However, the practical examination was abolished in 1982 and a new type of clinical test was introduced, that is a paper test to question the diagnosis or decisions of actual clinical cases and not to evaluate candidate's dental skills. Another revolutionary change of our dental license system was an introduction of the dental residency program in 2006. Almost at the same time, the common achievement test (CAT) consisted of computer-based testing (CBT) and objective structured clinical examination (OSCE) has been subjected to all dental students before starting general practice sessions. It is a national-based standard evaluation test of basic and clinical knowledge, skills, and attitude. Our CBT is nearly equivalent to the National Board Dental Examination (NBDE) Part I and Part II in the United States, and the OSCE tests attitude and clinical skills needed for the general practice sessions using simulated patients and dental simulators. Furthermore, it has reported that NED placed more emphasis on recall in cognitive level, but NBDE Part II are more focused on problem solving (Komabayashi & Bird, 2005), suggesting the improvement of NED to ask the deeper level of clinical knowledge.

Since the abolishment of practical exam in 1982, it has been discussed about the issue how we credit the clinical competency of dental students at their graduation. They can obtain dental license immediately after passing NED and start the residency program to treat patients without evaluating clinical skills and attitude acquired during general practice sessions. The U.S. candidates for dental license are subjected to the clinical examinations conducted by individual state boards of dentistry or regional dental testing agency. Currently, five such regional agencies develop and administer a standardized clinical examination working jointly with the state boards. However, the clinical competency exam (CCE) equivalent to U.S. dental license system has not been reintroduced in Japan NED till now.

The necessity for improvement of clinical training system for undergraduate students

In the year of 2009, the ad-hoc committee of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) released the preceding report entitled "Strategies for training of dentists with solid clinical competency". In this report, it was pointed out that general practice sessions in Japanese dental schools has lost their substances and failed to increase the clinical competency of students at the graduation, indicating the necessity for an immediate improvement of undergraduate clinical training system. International standardization is also required to Japanese dental education program, and

the gap of education quality existed between dental schools was expanded. All dental students took the common achievement test before starting general practice session in their dental schools and were supposed to reach a certain level in their clinical competency as well as knowledge. For equation of the quality of clinical education, a standardized CCE before graduation would be suitable to measure the clinical ability of students from multiple dental schools. It is also getting very hard to secure collaborative patients for student clinic after starting up of dental residency program, because patients suitable for dental residents are also good for undergraduate clinical training. The changes in educational environment lead to strong demands for an intelligent program for clinical training of undergraduate dental students.

PROGRAM FOR PROMOTING INTER-UNIVERSITY COLLABORATIVE EDUCATION

Sophistication of Dental Education Program Utilizing Inter-School Relationship: Roles of Tohoku University School of Dentistry

In the fund year of 2012, MEXT adopted the project named "Sophistication of Dental Education Program Utilizing Inter-School Relationship" as the Program for Promoting Inter-University Collaborative Education. This project has been carried out in collaboration of three national university dental schools: Niigata, Hiroshima, and Tohoku. The mission is to develop the more sophisticated training program of dentists to meet the social change and needs of the age, that are common issues of all Japanese dental schools. Under the developed training program, three dental schools will enforce the integrated dental skill education to their students and distribute it to other dental schools across the country in the future. Tohoku University School of Dentistry fulfills a role in the project by developing 3 types of programs: 1) cultivation program of dental researchers; 2) collaborative educational program of dentistry and engineering, and 3) advanced clinical practice training program. In the following chapters, we would like to focus on our last mission, development of advanced clinical training program.

Developing the sophisticated clinical training program: Advanced Dental Skills Lab Training

Fig. 1 summarized our proposed program of clinical training for undergraduate students. This program includes two new proposals to enhance and guarantee the clinical competency of students at the time of graduation. The first proposal is "Advanced Dental Skills Lab Training". Dental skills lab training has been already introduced in the general practice session of many dental schools, which usually includes trainings of teeth preparation and endodontic therapy using simulators (Fig. 2A). These trainings are effective to improve each dental skill of students necessary for general practice, but do not include training of attitude and skills for medical interview. Dental students train these attitude and skills from patients at the clinic. As the real patients are not standardized unlike simulated patients, it takes time to obtain enough information for diagnosis and treatment

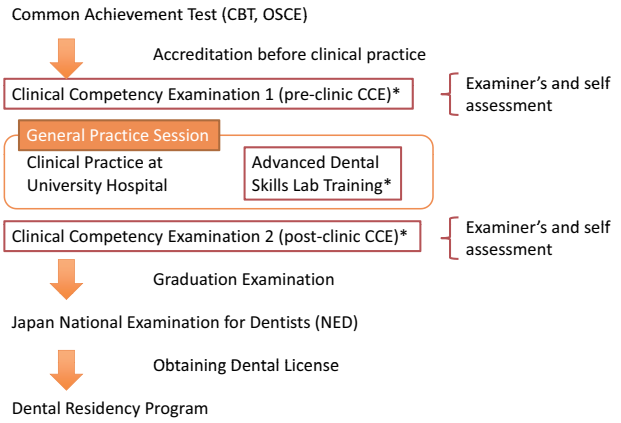

Fig. 1. Proposed Program of Clinical Training for Undergraduate Dental Students
*Program developing under this project.

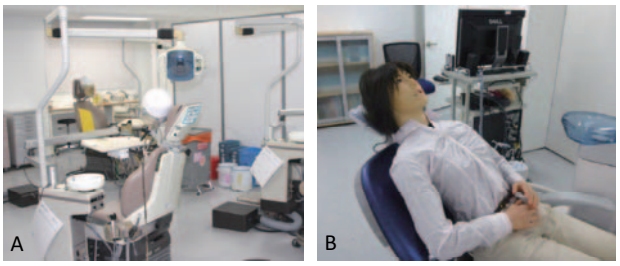

Fig. 2. Advanced dental skills lab
A. Usual skills lab for training of tooth preparation and endodontic therapy
B. Patient robot system (SIMROID®)

by student's interview. Only repeated trainings can resolve problem and improve their interview skills.

We recently introduced the humanoid patient robot system (SIMROID®, Morita Corp., Osaka, Japan) in the skills lab (Fig. 2B), which is a specially designed patient simulator developed as the learning tool for OSCE. This robot has three types of operation system: 1) computer manipulation by an operator; 2) interactive voice response system (IVRS), and 3) automated response system by a built-in sensor. IVRS can provoke a brief introduction and formulaic answers according to pre-programmed scenarios compatible to OSCE assignments. To apply this robot for clinical level training, computer-manipulated system with original scenarios would be suitable by assuming variable clinical situation and patient responses, *i.e.* a patient with systemic disease or medication to consider for decision-making. Unlike a typical auto-answering by the robot, human simulated patients can stratify the students' question level and respond appropriately according to their words. To learn the appropriate questions in individualized case, several patterns of answer were pre-installed for one question. The operator (instructor) selects one of them by judging the level of student's question and gives a command to robot. We're now developing scenarios with multiple patterns of answers simulated various clinical situations.

Developing the sophisticated clinical training program: Japanese version clinical competency examination (CCE)

Generally in many dental schools in Japan, graduation exam is held to test students' knowledge of basic and clinical dentistry. However, CCE is only introduced as paper clinical test in Japan NED, but dental schools usually do not test student's skills and attitude after finishing general practice session by objective way of confirming. Advanced OSCE before graduation has been suggested as an appropriate way except for the problems of its time and cost. In the U.S., CCEs are conducted by individual state boards of dentistry or by regional dental testing agencies and involve performing dental procedures on patients, and there may also be a laboratory or manikin component. On the other hand, Canadian NBDE subjects all candidates to take OSCE, which is almost similar to paper clinical test of Japanese NBDE, for application of dental license.

Considering that the Japanese dental education system has already incorporated OSCE before starting clinical session and paper-based clinical test at NBDE, we should think how we create the exam system to certificate students' clinical skills at their graduation. Using U.S. CCE as reference, we are conducting to develop manikin-based exam using an integrated jaw model. According to the dental exam guide of one U.S. agency (WREB 2013 Dental Exam Candidate Guide), their CCE is composed of five sections: Operative, Periodontics, prosthodontics and Patient assessment and Treatment planning. Manikin and jaw model are only used in the exam for endodontics, however our plan is to develop the jaw model to test operative, endodontic, periodontal, and prosthodontic procedures (Table 1). For the base of new jaw model, we selected the integrated practice model developed by our partner, Niigata University (Model: D16-NI. P22, Nissin, Tokyo, Japan) and try to convert it suitable for short-term examination and practice.

Table 1. Required elements for CCE testing clinical skills

Operative
Composite Resin Restoration (Cavity preparation, Filling)
Inlay Preparation (Class II cavity)
Endodontics
Root Canal Treatment (Access Opening, Canal shaping, Filling)
Periodontics
Scaling & Root planing (SRP)
Prosthodontics
Crown Preparation (Posterior Full Cast Crown)
Fixed Bridge Preparation (Anterior Facing Bridges)

Another feature of U.S. CCE system is that they disclose scoring criteria for all five components. We should also develop the original scoring criteria and system for our skills test components. Our scoring criteria are also disclosed to students and applied for their self-assessment. As shown in Fig.1, it is recommended that pre- and post-practice CCEs should be conducted to enable the formative assessment of students including both examiner's assessment and self-assessment by student. The humanoid patient robot system is also applicable for patient assessment test of skills in medical interview.

DISCUSSION

The primary mission of dental school is to educate dental students with high competency and to deliver high quality of dental service to the public. To accomplish this mission, we should go forward to improve the quality of education. We believe that our program entitled "Sophistication of Dental Education Program Utilizing Inter-School Relationship" will provide the strong momentum for this improvement by delivering the new clinical education program.

ACKNOWLEDGEMENT

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Cultivation of Bio-Dentists with Global Competency and Advanced Technology.

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INTRODUCTION

Faculty of Dentistry, Hiroshima University, has worked on advanced education, in corporation with Niigata University and Tohoku University, under the Program for Promoting Inter-University Collaborative Education provided by MEXT (Ministry of Education, Culture, Sports, Science and Technology). The missions of Hiroshima University are as follows, 1; To develop and provide the education program for internationalization and globalization, 2; To provide the program to send the students overseas for short periods, and 3; To provide the educational program for advanced dental technologies.

Besides this, in the last decade, we developed the strategic triad of education, comprising Bio-dental education, Inter-professional education and International education.

In the session, we would like to introduce our strategic triad of education in relation to our missions.

1. BACKGROUND

Faculty of Dentistry, Hiroshima University was established in 1965, and that environment surrounding the dentistry was that he shortage of dental practitioner due to much full dental caries and missing teeth. However, during these 40 years, the remarkable changes in disease structure occurred with the rapid aging of the population. Hence the focus of education in our faculty should be changed from the accumulation of knowledge to the voluntary and creative education. It seems to constitute the dental education by two parts: innovation and foundation. The foundational part guarantees the educational quality, namely the medical quality in the dentistry education, and the innovative part contributes to the advance of dentistry. The innovative part becomes established knowledge and technology in the long term, and it shifts to the foundational education and results in the improvement in the medical quality. That is to say, it is considered that the advanced research is indispensable to the advanced education. These backgrounds of present dentistry and society encouraged Hiroshima University Faculty of Dentistry to reorganize undergraduate education system into two different education courses, the frontier dental science course and the advanced dental clinician course, in school of dentistry in 2000.

Hiroshima University Faculty of Dentistry had School for Dental Hygienists, established in 1976, and Dental Technicians School established in 1972. These two schools are integrated and reorganized to the School of Oral Health Science in 2005. The four-year pro- gram for dental hygienists was firstly introduced at Tokyo Medical and Dental University and Niigata University in 2004 in Japan. In 2005, Hiroshima University firstly established the School of Oral Health Science with four-year programs for dental hygienists and dental technicians in Japan. In the bachelor program, we cultivate either the dental hygienists or dental technicians to have the capability for research. The capability of research is most important to chart the future of dental hygienist and/or dental technicians.

2. BIO-DENTAL EDUCATION

Through these 3 bachelor programs, we cultivate the bio-dentists, oral health manager, and oral engineer, respectively, and three both have the potential to advance the research and develop the frontier field of the medical and dental researches based upon the biology and engineering. Excellent and innovative course works derived from 3 programs, were carefully selected, and Bio-dental education, which is an inter-professional education, was started in 2010, supported by MEXT. Bio-dental education comprises 1) Start-up course work, 2) Advanced course work and 3) Practical English course. The Start-up course work comprises Basic practice for cell culture, Practice for CAD system engineering and Practice for Medical Equipment, and provided to the 3rd grade students of School of Dentistry, and 2nd grade students of School of Oral Health Sciences, incl. courses for both Oral Health Science and Oral Engineering. All the students take Start-up Course Work.

Advanced Course Work comprises Practice for Oral Infection, Practice for Clinical Diagnosis, Practice for Dental Regeneration, and Practice for evaluation of oral function. Advanced Course Work and Practical English Course are provided to the 4th grade students of School of Dentistry, and 3rd grade students of School of Oral Health Sciences, incl. courses for both Oral Health Science and Oral Engineering. All the students take Practical English Course, but the Advanced Course Work is elective, and the students choose the one from four Practices described above.

We provide the bio-dental program as the leading program for dental technologies in the Program for Promoting Inter-University Collaborative Education.

3. INTERNATIONAL DENTAL COURSE

International Dental Course with Lectures conducted in both English and Japanese was started in April 2012. Three students from 3 universities, i.e. University

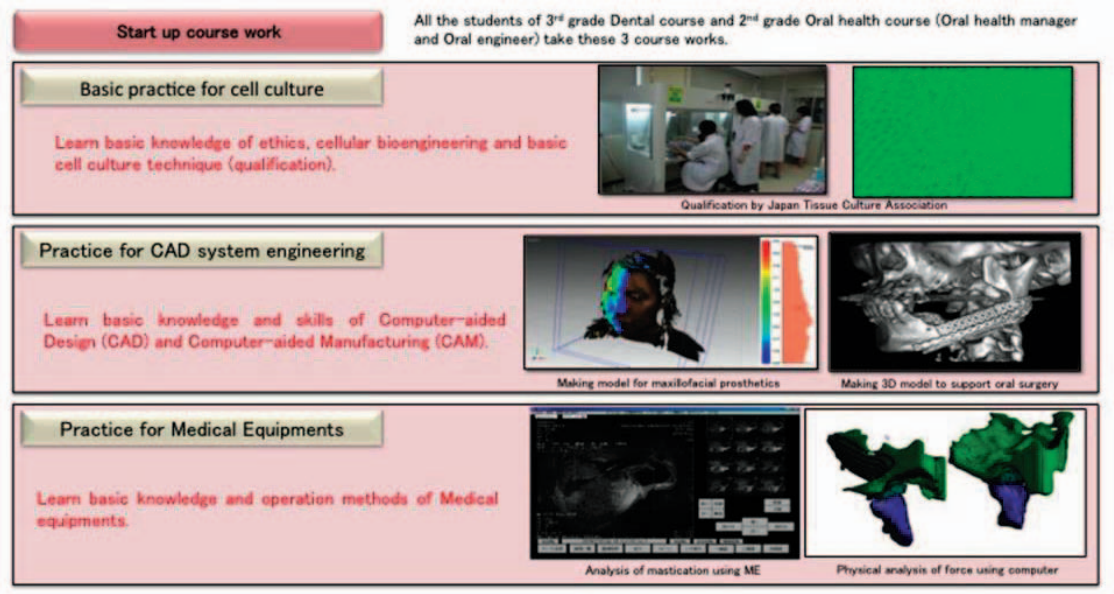

Fig. 1. Start-up Course of Bio-dental Education
All the students of 3rd grade Dental course and 2nd grade Oral health course (Oral health manager and Oral engineer) take these 3 course works.

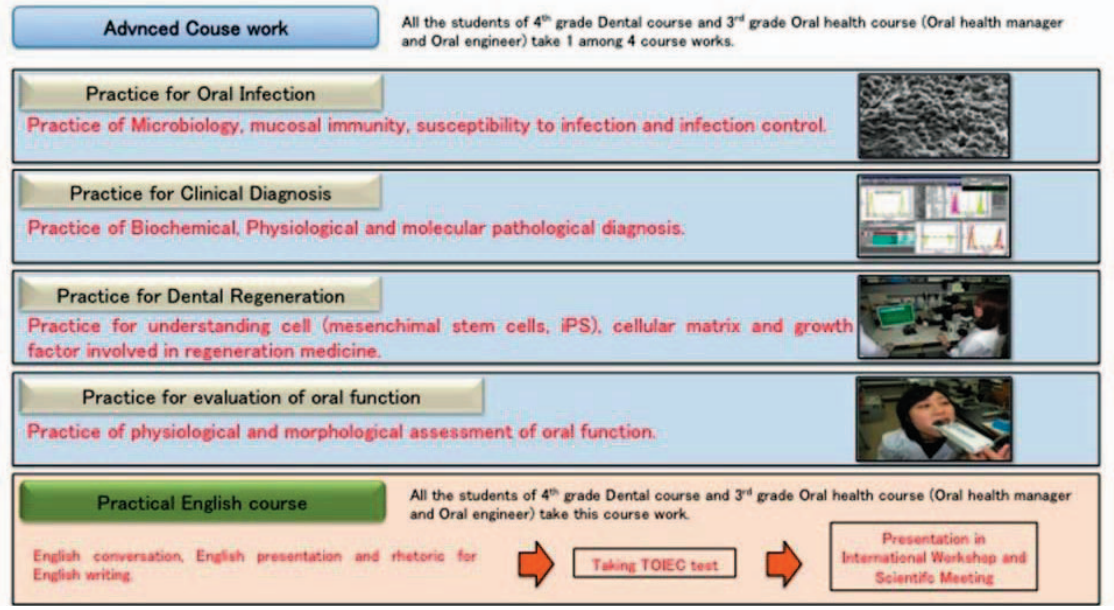

Fig. 2. Advanced Course of Bio-dental Education
All the students of 4th grade Dental course and 3rd grade Oral health course (Oral health manager and Oral engineer) take 1 among 4 course works

of Airlangga, Indonesia, University of Health at Hochiminh City, Vietnam and University of Health Science, Cambodia, are received in the school of dentistry every year. All the students have completed the cultural education for one year in their own countries. After acceptance, they study Japanese language and culture and Basic Biology for 6 months before the professional education starts. Subsequently, They learn Basic Subjects such as Anatomy, Physiology, Biochemistry, Pathology, Microbiology, Immunology..., Clinical Subjects and Common Core Programs in relation to dentistry for four years. After the completion of our curriculum, they will return to their own countries, to do the Undergraduate clinical practice in home country.

Department of International Collaboration Development for Dentistry (ICDD), plays an important role in planning or driving the International Dental Course.

ICDD was established in 2011, in advance to the start of International Dental Course. The chief of the ICDD is Professor Takata, who started the International Dental Course under the support of MEXT. ICDD manages and supports the International Dental Course, and also develop the short exchange programs for students. The contribution of ICDD to the exchange programs, the number of student participants to Short-term stay (SS) program and

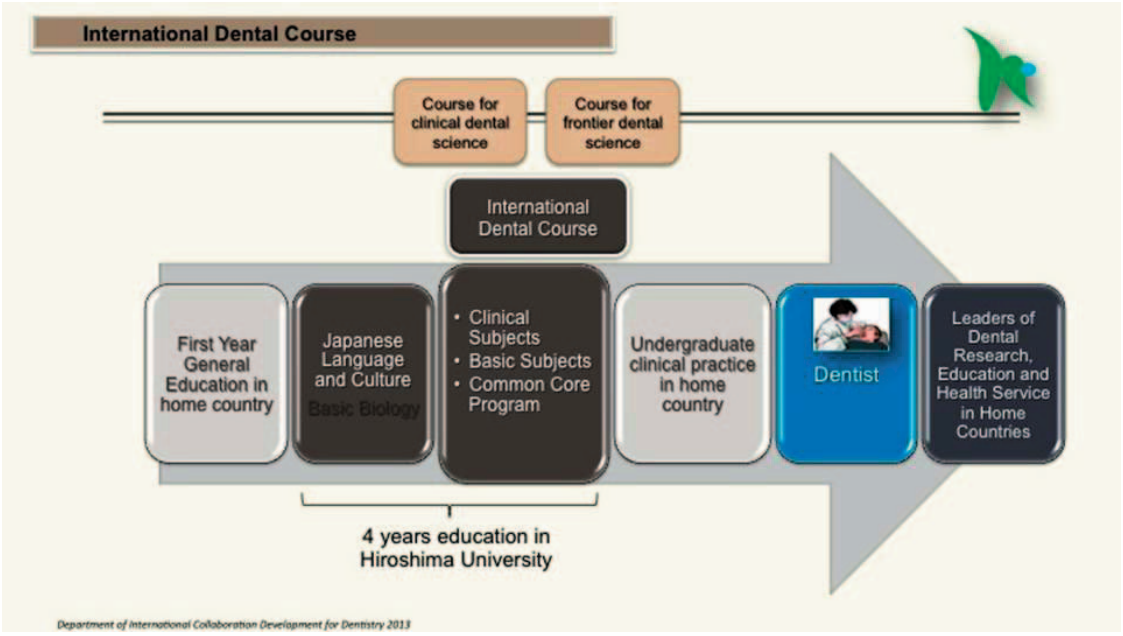

Fig. 3. Summary of International Dental Course

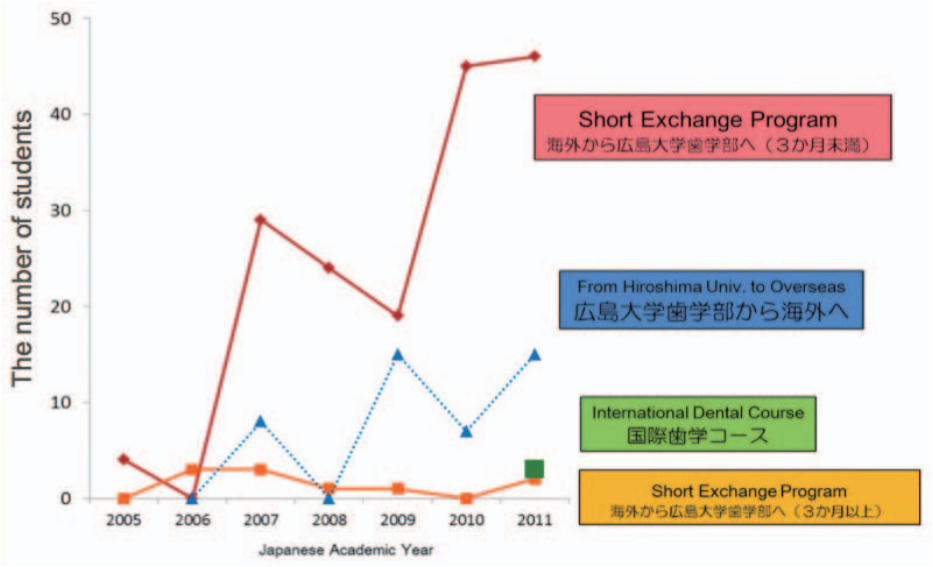

Fig. 4. Changes in the number of student participants in international exchange program.

Short-term visit (SV) program dramatically increased.

Hence, through the lectures in International Dental Couse and ICDD, we can provide the International education program and Short stay/short visit program in the Program for Promoting Inter-University Collaborative Education.

Clinical Education of Dental Practice at University of Washington

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Key words: accreditation, competency, comprehensive care, patient-centered care

ABSTRACT

In this brief presentation, I will relate my experience as to how the UWSoD dealt with the evolution of patient-centered care, quality improvement approaches, and informatics. The three topics will be presented in the context of how we continue to cope with changes with the leadership and visions of our new Dean. The last part of the presentation will deal with the future of dental clinical education at the University of Washington.

All health professional should be educated to deliver patient-centered care as members of an interdisciplinary team, emphasizing evidenced-based practice, quality improvement approaches, and informatics—Quality Chasm report (Institute of Medicine, 2001)

INTRODUCTION

The Workshop organizing committee charged me to speak on "Clinical Education of Dental Practice at University of Washington and USA". I have taken the liberty to make a slight change and remove "USA" from the title. The scope is much too broad to be covered in a short talk and workshop paper. Although I'll certainly touch on dental education in the USA in general, I'll focus mainly on my responsibilities as Associate Dean for Clinical Services and as a Professor of Restorative Dentistry at the University of Washington, School of Dentistry (UWSoD).

I will address "Clinical Education of Dental Practice", in the context of how we teach our pre-doctoral dental students to properly handle patient care. UWSoD has the advantage of being one of six Health Science schools on the University of Washington campus. The six Health Science Deans meet regularly to discuss matters related to interdisciplinary professional education. Although we are trained differently in our disciplines, the core attributes of patient-centered care, evidenced-based practice, quality improvement approaches, and informatics run in all six Health Science Schools. As such, I'll approach the education of our dental students from a perspective of a dental or healthcare professional.

The University of Washington (UW) was ranked eighth among public universities in the United States in the quality of undergraduate education, according to Forbes (July 2013). UW was ranked first among all colleges and universities in Washington. Our UWSoD has also been ranked in the top tiers among US dental schools consistently. Although we are very proud of our strong clinical tradition, we are aware of the impending changes in dental and medical education. We must continue to evolve as a dental school in order to thrive.

In this brief presentation, I will relate my experience as to how the UWSoD dealt with the evolution of patient-centered care, quality improvement approaches, and informatics. The three topics will be presented in the context of how we continue to cope with changes with the leadership and visions of our new Dean.

The last part of the presentation will deal with the future of dental clinical education at the University of Washington.

CODA ACCREDITATION

My story started around September, 2008 when I first joined University of Washington as a faculty member. Before I begin, it is important to explain the dental school accreditation process, especially as it relates to patient care. Dental and dental-related education programs conducted at the post-secondary level are accredited by the Commission on Dental Accreditation (CODA), which was established in 1975. CODA employs a collaborative peer review accreditation process to evaluate the quality of over 1,300 dental education programs nationwide, including dental schools, specialty programs, clinical fellowships and allied dental training programs. The principal aim is to maintain the highest professional and ethical standards in the nation's dental schools and programs. CODA is nationally recognized by the United States Department of Education (USDE).

Since the early 1980s, accreditation standards for dental school have included patient care and clinic management. The standard that refers to patient care and clinic management has undergone several changes. At its January 31, 2013 meeting, the Commission adopted the revised Accreditation Standards for Dental Assisting Education Programs, Advanced Specialty Education Programs in Endodontics, Orthodontics, Periodontics,

Dental Public Health and Oral and Maxillofacial Pathology. The revised standards and self-study guides will be implemented January 1, 2014. These same guidelines will be used to evaluate UWSoD in 2016.

The accreditation process begins when a sponsoring institution submits an application to CODA. The institution then completes a comprehensive self-analysis and self-study report detailing its resources, curriculum, policies and operational standards: (*www.ada.org/sections/educationAndCareers/docs/pde_ssg_2013.doc*). The self-study is intended to involve all the communities within the institution in an internal examination of the ways in which the institution and its programs meet its own stated purposes and the accreditation standards approved by the Commission. It is the self-study process that introduced me to the UWSoD.

SELF-STUDY & SWOT ANALYSIS

A month before I officially joined the faculty, I was invited to participate in the school's summer faculty retreat and discussion regarding their finding of a self-study in preparation for the upcoming accreditation process. All accredited dental programs receive a site review every seven years, except for programs in the specialty of oral and maxillofacial surgery, which are reviewed every five years. UWSoD's last accreditation process was scheduled to be reviewed in July, 2009. When I arrived in Seattle, Washington, the self-study process had already begun in earnest. I was assigned to be Co-chair of Standard 5, Patient Care Services. We had a little over a year to prepare for the site visit.

The school's self-study report, in the form of a SWOT analysis, detailed the school's resources, curriculum, policies and operational standards. SWOT analysis is a structured planning method used to evaluate the **S**trengths, **W**eaknesses, **O**pportunities, and **T**hreats involved in a project, in this case, to successfully prepare for the upcoming accreditation. Identification of SWOTs is important because they can inform later steps in planning to achieve the objective. One of my first assignment as Associate Dean for Clinical Services was to prepare the School to remedy some of the weaknesses identified.

UWSoD faculty identified patient-centered comprehensive care as a major area needing attention in order to address accreditation standards (Table I):

Rank 1. Standard 5-2: (Comprehensive care)—No mechanism in place for verifying treatment is complete or QA check as patients complete treatment at UWSoD.

Rank 2. Standard 2-25: Comprehensive Treatment Planning and Risk Assessment

Rank 3. Standard 2-16: Patient-centered care is not reinforced through all the undergraduate clinics and is undermined by the emphasis on technical requirements

Rank 5. Standard 2.5, 2.6, 2.7, 2.10: Curriculum innovation—including Comprehensive care.

As one can see, comprehensive and patient-centered care was high in the overall ranking and needed immediate attention.

COMPREHENSIVE CARE MODEL

In Oct 2008, soon after the August faculty retreat, the Dean appointed a Comprehensive Care Task Force comprising of six representatives from three clinical departments and the Dean's office. I was appointed as the Chair of the task Force. Before we started, it would suit us well by looking at the comprehensive care model of

Table I. CODA STANDARD 5 - PATIENT CARE SERVICES (prior to Dec 2013)

5-1 The dental school **must** have a published policy addressing the meaning of and commitment to patient-centered care and distribute the written policy to each student, faculty, staff, and patient.

5-2 Patient care **must** be evidenced-based, integrating the best research evidence and patient values.

5-3 The dental school **must** conduct a formal system of continuous quality improvement for the patient care program that demonstrates evidence of:

a. standards of care that are patient-centered, focused on comprehensive care and written in a format that facilitates assessment with measurable criteria;

b. an ongoing review and analysis of compliance with the defined standards of care;

c. an ongoing review of a representative sample of patients and patient records to assess the appropriateness, necessity and quality of the care provided;

d. mechanisms to determine the cause(s) of treatment deficiencies; and

e. implementation of corrective measures as appropriate.

5-4 The use of quantitative criteria for student advancement and graduation **must** not compromise the delivery of comprehensive patient care.

5-5 The dental school **must** ensure that active patients have access to professional services at all times for the management of dental emergencies.

5-6 All students, faculty and support staff involved in the direct provision of patient care **must** be continuously certified in basic life support (B.L.S.), including cardiopulmonary resuscitation, and be able to manage common medical emergencies.

5-7 Written policies and procedures **must** be in place to ensure the safe use of ionizing radiation, which include criteria for patient selection, frequency of exposing radiographs on patients, and retaking radiographs consistent with current, accepted dental practice.

5-8 The dental school **must** establish and enforce a mechanism to ensure adequate preclinical/clinical/laboratory asepsis, infection and biohazard control, and disposal of hazardous waste.

5-9 The School's policies and procedures **must** ensure that the confidentiality of information pertaining to the health status of each individual patient is strictly maintained.

clinical education, according to the IOM report, which is characterized by the following attributes:

- A generalist role model rather than a specialist role model,
- Patient-centered education rather than a student centered education,
- Continuity of patient care rather than segmented patient care,
- A focus on evaluation and management rather than a procedure focus, and
- Competency criteria rather than numerical requirements.

With these guidelines in mind, a proposal was reported to the Dean on Feb 23rd, 2009 and also to the Faculty Council and Executive Committee in May 2009. A pilot version of the Comprehensive Care model in the form of Student Advising/Patient Treatment Management was spearheaded by the Restorative Department and was implemented in June 2009. The pilot program ensures that 1) students learn how to plan, implement and manage treatment of more complex cases, 2) patient treatment is planned and sequenced properly, and approached comprehensively and with continuity 3) clinical competency is achieved in the areas of patient treatment planning and informed consent for treatment, and 4) feedback and input from students and faculty can better prepare the final implementation of the Comprehensive Care Model.

The Student Advising/Patient Treatment Management Process has many of the features of the proposed Comprehensive Care Model. Briefly, Restorative faculties are organized into 5 faculty advising groups with three or more faculty per group. Each group is responsible for approximately 13 third-year and 11 fourth-year students. Faculty from other departments, namely Endodontics, Periodontics, Oral Surgery Oral Medicine and Orthodontic, will act as consultants. It was anticipated that in the future second and first-year students could be incorporated into the system.

Although the model is still a work in progress, I am happy to report that currently UWSoD provides an integrated and comprehensive clinical curriculum of patient experiences to all predoctoral students. Patient care experiences fall into two main categories: (1) comprehensive care of patients and (2) clinical rotations to "dental specialty" clinics (e.g., Endodontics, Oral Medicine Emergency Clinic, Oral Surgery, Orthodontics, Pediatric Dentistry, Dental Fears Clinic) and hospitals affiliated with the University of Washington Academic Medical Center (UWAMC).

Our present Dean, Dr. Joel Berg, has visions to revamp our current system to meet the needs of the future. Our strategy is to start the patient encounter experience earlier and adopt the clerkship model. He has created several Task Forces to deal with the many issues. This will be described in greater detail in the last section.

PATIENT CARE ENCOUNTERS

The following is a summary of patient care encounters in the UWSoD (excerpts from our 2009 accreditation report):

First Year

Early patient care experience begins in the second quarter of the curriculum. Students receive instruction on infection control, prevention, dental record keeping and professionalism in addition to participation in clinical sessions in the Oral Medicine Clinic as a part of "Introduction to Clinical Dentistry". During these sessions, students learn introductory clinical skills necessary to work with patients in a clinical setting and have the opportunity to make clinical correlations with knowledge gained in the biological sciences curriculum.

Second Year

Early patient and clinical experience continues in the fifth quarter, when students are assigned a complete denture patient through the Department of Prosthodontics. Students perform a clinical interview of their denture patient, reviewing the patient's medical history (including nutritional analysis) and dental history. Periodontic patient care experiences begin in the sixth quarter with the PERIO Prevention/Periodontics series. Students learn initial periodontic treatment, including medical health history, periodontal examination, dental prophylaxis, scaling, root planing, and coronal polishing. In the eighth quarter, students begin treatment planning for comprehensive care patients. Students also observe the comprehensive examination of patients during rotations in the Oral Medicine Clinic at the end of the second year.

Third Year

At the beginning of the third year, patient care experiences become more prominent in the curriculum. Student-based patient care experiences are structured by the need to obtain clinical competence through the repetition of specific procedures. There are opportunities to achieve clinical competence through patient care experiences in the Comprehensive Care Clinics as well as during required rotations in specialty clinical settings within the school and affiliated clinics. By the end of the third year, students have completed a total of eight rotations of patient care in the Comprehensive Care Clinics with the Department of Restorative Dentistry. Students also are assigned to three rotations in the Department of Periodontics and three rotations in the Department of Prosthodontics.

Students are required to complete eight rotations with the Department of Oral Medicine. During these rotations, students treat patients in the Oral Medicine Emergency Clinic and perform the initial comprehensive examinations of entering patients. While rotating through the department's DECOD (Dental Education in Care of Persons with Disabilities) Clinic, students learn firsthand about the unique oral health needs of a variety of disabled patients and provide these patients with comprehensive dental care. Oral Medicine rotations also include working off-site with faculty treating patients with cerebral palsy at the Provail Clinic.

Third-year students also complete two rotations each in the Departments of Endodontics, Oral and Maxillofacial Surgery, Orthodontics, and Pediatric Dentistry. During these rotations, students work with faculty, residents, and graduate students to provide

patient care in each department's clinic. Instruction in these clinical rotations focus on preparing students to achieve the school's core entry-level competencies.

Fourth Year

Beginning in the 12th quarter (third year) and extending into the fourth year, students continue with patient experiences in the Comprehensive Care Clinics. Additional rotations are added in off-site and specialty locations. Students are required to complete a rotation at one of two nursing home facilities. Students also complete a hospital rotation consisting of a week of clinical patient contact at Harborview Medical Center, Children's Hospital and Regional Medical Center, the UWAMC, or a combination thereof. Each student is required to be on-call at the UWAMC Emergency Clinic five to seven times as part of this rotation. Each student is also required to attend one session in the Dental Fears Clinic through the Department of Dental Public Health Sciences. Fourth-year students are scheduled for a total of 12 rotations of comprehensive care in the Department of Restorative Dentistry and three rotations each in the Departments of Periodontics and Prosthodontics.

Fourth-year students continue endodontic patient care and complete a minimum of three rotations in the Endodontic Clinic. Students can use additional time during their fourth year to complete any outstanding rotations with Pediatric Dentistry and Oral and Maxillofacial Surgery, which should be completed by the end of quarter 13.

In addition to acquiring the required patient care experiences in the Comprehensive Care Clinics, the specialty clinics, and off-site rotations, students must complete at least two elective courses (minimally, one during the third and one during the fourth year). The School of Dentistry offers many clinical and honors elective rotations in the specialty clinics, allowing students to gain valuable patient experiences in areas of student interest. Offerings include dental implant training, advanced rotations in treating disabled patients in the DECOD Clinic, cast and direct gold restorations, intravenous sedation, and directed studies in the specialty clinics.

Typically, UWSoD has around 70,000 patient visits per year. We experienced a slight decrease in patients seeking treatment in the dental school around 2009. Such a downturn coincided with the economic depression nationally and the termination of adult Medicaid. The School implemented various short-term measures to maintain the influx of patient, including a bus-advertisement campaign and a Community Dental Program aiming to help the Medicaid population. Currently we are seeing a slow return to the previous patient level with the economic condition improving.

In summary, the patient pool is more than adequate to provide patient care experience of sufficient scope, variety, and number to allow all students to achieve the core competencies in a timely manner.

COMPETENCY CRITERIA —HEALTH AND DENTAL PROFESSIONAL HEALTH PROFESSIONAL

The Institute of Medicine published a report in 2003 entitled: Health Professions Education: A Bridge to Quality. This report is the result of an interdisciplinary summit held to identify core competencies required of 21st century healthcare professionals in order for them to be prepared to deliver quality patient care and assure patient safety.

The report identified five core competencies to be embedded in all health professions curriculum: 1) Provide patient-centered care, 2) Work in interdisciplinary teams, 3) Employ evidence-based practice, 4) Apply quality improvement and 5) Utilize informatics. These are the core competencies that are needed in the graduates of our programs in order for them to serve the needs of patients and healthcare organizations as they reshape the healthcare system to achieve the quality, safety and efficiency imperatives placed upon them by the American society in the 21st century and beyond.

Dental professional

While the UWSoD has specific core competencies for our graduating dental students (Table II), each department has its own set of competencies before they declare that a student can graduate. Since I am a faculty in the Restorative department, I'll use Restorative's example to illustrate the point.

Restorative Dentistry has seven criteria of clinical competencies which all students must challenge individually and pass before graduation. The competency examinations are part of their clinical grades.

Before they enter into the fourth year, i.e. the summer quarter of their third year, all students must complete the following four competencies:

1. Restorative Dentistry Treatment Planning
2. Operative dentistry—Class 3 Composite Restoration
3. Operative dentistry—Class 2 Composite Restoration
4. Operative dentistry—Class 2 Amalgam Restoration

The following three competencies are to be completed before the June graduation:

1. Laboratory Quality Control—Porcelain-Fused-to-Metal crown, Complete Ceramic Crown or Full Gold Crown Restorations
2. Fixed Prosthodontics—Porcelain-Fused to-Metal crown, Complete Ceramic Crown or Full Gold Crown Restorations
3. Fixed Prosthodontics—Complete Implant Crown

An example of the Class 3 Composite Restoration competency is illustrated in Figure 1. Other clinical departments run their own competencies in a similar manner.

QUALITY IMPROVEMENT APPROACHES

Aside from the focus of patient-centered care, my first and foremost job as Associate Dean for Clinical Services is to provide a confidential and safe environment for learning, while utilizing appropriate technology and techniques. One of the major accomplishments, since the time I first took over the job in 2008, was to make sure that we have a Coordinated Quality Improvement Plan (CQIP). With the help of our Medical School and Risk Management colleagues, we were able to finalize and register our CQIP plan with the Washington Department of Health and have it approved in 2009.

Table II. LIST OF UWSoD COMPETENCIES

UWSOD Competency 1:
Examine a patient using contemporary diagnostic methods to evaluate the head and neck region and to reach a diagnosis of the patient's oral and craniofacial health status.

UWSOD Competency 2:
Communicate the risks and benefits of proposed care and alternative treatment strategies available, then obtain expressed consent for treatment conveyed by a patient or legal guardian.

UWSOD Competency 3:
Formulate a comprehensive treatment plan based on diagnostic findings, then implement treatment in a safe, properly sequenced and timely manner.

UWSOD Competency 4:
Provide patient education in the prevention of oral diseases to promote oral and general health.

UWSOD Competency 5:
Recognize the limits of their expertise and seek consultation with other health care providers to facilitate patient care.

UWSOD Competency 6:
Manage acute and chronic orofacial and dental pain.

UWSOD Competency 7:
Manage pulpal and periradicular disease.

UWSOD Competency 8:
Manage dental emergencies.

UWSOD Competency 9:
Manage medical emergencies in dental practice by providing basic life support.

UWSOD Competency 10:
Prescribe and administer pharmacological agents for patient care.

UWSOD Competency 11:
Diagnose and manage hard and soft tissue lesions and diseases of the orofacial complex.

UWSOD Competency 12:
Perform uncomplicated oral surgical procedures.

UWSOD Competency 13:
Assess the dentition to determine the need for orthodontic treatment.

UWSOD Competency 14:
Manage periodontal diseases.

UWSOD Competency 15:
Assess the teeth and supporting structures and provide preventive services.

UWSOD Competency 16:
Manage diseases and conditions of the teeth.

UWSOD Competency 17:
Manage replacement of teeth for the partially or completely edentulous patient.

UWSOD Competency 18:
Practice dentistry within the ethical standards of the dental profession and the law.

UWSOD Competency 19:
Utilize information-technology resources in contemporary dental practice.

UWSOD Competency 20:
Utilize business and management skills to conduct an efficient and effective clinical practice.

UWSOD Competency 21:
Recognize the role of lifelong learning and self-assessment in maintaining competency.

UWSOD Competency 22:
Utilize critical thinking in assessing technical and scientific information for use in identifying patient needs and treatments.

UWSOD Competency 23:
Apply the principles of behavioral science that pertain to patient-centered oral health care.

UWSOD Competency 24:
Evaluate different models of oral health care management and delivery.

UWSOD Competency 25:
Manage a diverse patient population and have the interpersonal and communication skills to function successfully in a multicultural work environment.

UWSOD Competency 26:
Evaluate the outcomes of treatment.

Once the CQIP was in place, we were able to carry out the following objectives:

1) Improve the health outcomes of the school's patients through the clinical care delivered by faculty, residents and students.
2) Ensure a safe and healthy environment of care for patients, employees and staff.
3) Monitor and improve, if necessary, patient satisfaction with the clinical care and treatments they receive.
4) Facilitate training and other educational activities for faculty and students so that they understand the legal aspects of providing health care.
5) Oversee the process of peer review by which faculty receive meaningful feedback to improve their clinical proficiency.
6) Ensure policies and procedures are kept current.

I serve on both the CQIP Oversight Committee and the CQIP Operations Committee as Chair. The CQIP protocol put in place since 2009 has been put to the test several times over the past 4 years. It proved to be of great help in improving the overall quality of patient care. The root cause analysis fashioned after the Medical School model was revealing and educational.

INFORMATICS

Other improvements that I supported were the school's transformation to electronic health records (axiUm) and digital radiography. AxiUm allows our School to automate and streamline workflow and accounting, and to improve safety through quality management and outcomes reporting. More importantly, the searchable Electronic Health Record can increase the quality of care and promote evidence-based practice. As an example, with accurate information in our system, we can more efficiently participate in the Ryan White project on HIV patients and other multi-center clinical trials.

From the beginning, it has been our goal that we will

Clinical Competency Examination
Operative Dentistry
Class 3 Composite Restoration

EVALUATION SHEET

Student name _____ Tooth #_____
Examiner _____ Date _____ Surface_____

Preparation	S	U
Permission to Proceed		
♦Case selection meets criteria		
Outline and Extensions		
Proximal extension appropriate		
Gingival extension appropriate		
Bevels properly created if indicated		
Internal Form		
Pulpal depth appropriate		
Axial depth appropriate		
♦Retention/Resistance adequate		
♦Caries removal complete		
Pulpal protection (if needed and suggested by student)		
♦Pulpal exposure created; unavoidable; managed properly		
Smooth walls and margins		
♦Integrity of remaining enamel walls		
Operative Environment		
Adequate field – extent and fluid control		
♦Soft tissue management		
♦Adjacent teeth damage		
Finished Restoration		
♦Finished to margin; facial / lingual		
♦Gingival margin; smooth / no overhangs / no voids		
Contours compatible with adjacent		
♦Proper proximal contact area – resistance with floss		
Embrasures		
Anatomical form		
♦Smooth/polished surface; pitting		
Occlusion adjusted		
♦Procedure completed within time limit		
♦Patient satisfaction with shade		
♦Overall patient management		

S = Satisfactory U = Unsatisfactory * = Critical step

Examination Results: (initialed or stamped) **Pass**_____ **Fail**_____
REASON(S) FOR FAILURE

Figure 1. An example of the Clinical Competency Examination —Class 3 Composite Restoration Competency

no longer use the paper records/charts two years after the launching of axiUm. The paper record collection was stored off-site after July, 2011. Request of records can only be accommodated with advance notice.

The use of axiUm CE makes the School eligible for federal Electronic Health Record incentive funding. We anticipate receiving the federal funding in 2014. We also have a plan to ensure that the incoming first year students (2013) are connected to the central electronic health records via an issued lap-top for training purposes.

THE DENTIST OF THE FUTURE

In April 2008, the American Dental Education Association (ADEA) House of Delegates approved "Competencies for the New General Dentist" (Table III). ADEA is the voice of dental education. Members include all U.S. and Canadian dental schools and many allied dental education programs.

The purpose of this document and the proposed foundation knowledge concepts are to:

- Define the competencies necessary for entry into the dental profession as a general dentist. Competencies must be relevant and important to the patient care responsibilities of the general dentist, directly linked to the oral health care needs of the public, and must be realistic, and understandable by other health care professionals.
- Reflect (in contrast to the 1997 competencies) the 2002 Institute of Medicine core set of competencies for enhancing patient care quality and safety, and illustrate current and emerging trends in the dental prac-

Table III. Competencies for the New General Dentist - As approved by the ADEA House of Delegates on April 2, 2008

6. Patient Care

A. Assessment, Diagnosis, and Treatment Planning Graduates must be competent to:

6.1 Manage the oral health care of the infant, child, adolescent, and adult, as well as the unique needs of women, geriatric and special needs patients.
6.2 Prevent, identify, and manage trauma, oral diseases, and other disorders.
6.3 Obtain and interpret patient/medical data, including a thorough intra/extra oral examination, and use these findings to accurately assess and manage all patients.
6.4 Select, obtain, and interpret diagnostic images for the individual patient.
6.5 Recognize the manifestations of systemic disease and how the disease and its management may affect the delivery of dental care.
6.6 Formulate a comprehensive diagnosis, treatment, and/or referral plan for the management of patients.

B. Establishment and Maintenance of Oral Health Graduates must be competent to:

6.7 Utilize universal infection control guidelines for all clinical procedures.
6.8 Prevent, diagnose, and manage pain and anxiety in the dental patient.
6.9 Prevent, diagnose, and manage temporomandibular disorders.
6.10 Prevent, diagnose, and manage periodontal diseases.
6.11 Develop and implement strategies for the clinical assessment and management of caries.
6.12 Manage restorative procedures that preserve tooth structure, replace missing or defective tooth structure, maintain function, are esthetic, and promote soft and hard tissue health.
6.13 Diagnose and manage developmental or acquired occlusal abnormalities.
6.14 Manage the replacement of teeth for the partially or completely edentulous patient.
6.15 Diagnose, identify, and manage pulpal and periradicular diseases.
6.16 Diagnose and manage oral surgical treatment needs.
6.17 Prevent, recognize, and manage medical and dental emergencies.
6.18 Recognize and manage patient abuse and/or neglect.
6.19 Recognize and manage substance abuse.
6.20 Evaluate outcomes of comprehensive dental care.
6.21 Diagnose, identify, and manage oral mucosal and osseous diseases.

tice environment. These core competencies are divided into domains, are broader and less prescriptive in nature, are fewer in number, and most importantly will be linked to requisite foundation knowledge and skills.
- Serve as a central resource, both nationally for ADEA and locally for individual dental schools, to promote change and innovation in predoctoral dental school curricula.
- Inform and recommend to the Commission on Dental Accreditation standards for predoctoral dental education.
- Provide a framework for the change, innovation, and construction of national dental examinations, including those provided through the Joint Commission on National Dental Examinations and clinical testing agencies.
- Assist the development of curriculum guidelines, both nationally for ADEA and locally for individual dental schools, for both foundation knowledge and clinical instruction.
- Provide methods for assessing competencies for the general dentist.
- Through periodic review and update, serve as a document for benchmarking, best practice, and inter-professional collaboration and additionally, as a mechanism to inform educators in other health care professions about curricular priorities of dental education and entry-level competencies of general dentists.

UWSoD TASK FORCES

Dr. Joel Berg, DDS, MS, began his tenure as Dean of UWSoD in August 2012. One of his visions is for the UWSoD to educate and produce a dentist for the year 2025. In large part, this is dictated by the changes in dental and medical education and in dental accreditation standards (Table III). There is a trend toward increase inter-professional education. Together with the rest of the Health Science Deans, Dr. Berg is supporting the university-wide Inter-professional Education Initiative that helps to sustain Deans' commitment to transforming UW Health Science Education. This strategic vision is "to create at the University of Washington an integrated, collaborative learning system across the health and related professions that connects disciplines, promotes teamwork, fosters mutual understanding, strengthens research, and advances health for individuals and populations."

There is also a trend toward even more emphasis on evidence-based dentistry and ethical standards. Towards this end, Dr. Berg has created several Task Forces to tackle top issues. The following are excerpts from Dr. Berg's Dean's Update dated 6/7-2013:

Curriculum Renovation (led by Dr. Wendy Mouradian):

Now renamed the Curriculum Steering Committee, this group has completed its first phase of analysis and reporting. Following their recommendation, I have appointed a Curriculum Working Group, headed by Dr. John Evans of Oral and Maxillofacial Surgery, to lead the next phase of curriculum development. In addition to departmental and disciplinary expertise, the group includes faculty with particular expertise in areas across the entire curriculum, such as critical thinking and lifelong learning, communication skills and cultural competence, as well as ethics and competencies in interprofessional collaboration and leadership of the oral health team. Some of these will be grouped under an expanded Patient-Centered Practice Management Curriculum that will span all four years of pre-doctoral education. The Steering Committee has also been working closely with a special Interprofessional Education (IPE) Implementation Committee, convened by the Health Sciences Board of Deans, to develop IPE curricular content across the UW Health Sciences schools. Among other things, a proposal for including dental students and residents in a series of interprofessional curricular activities for academic year 2013-14 is now under consideration.

Clinical Systems (led by Dr. John Sorensen):

Starting with a comprehensive value stream mapping process last fall, this task force has exhaustively broken down the process of patient intake and treatment to identify inefficiencies and opportunities for improvement. (To get a firsthand view of the process, Dr. Sorensen even posed as a patient.) Now the task force is focusing its discussions on the adoption of a "clerkship" system of clinical rotations that would give our students a more intensive exposure to all the core competencies of general dentistry. The group has also focused heavily on the patient intake process—how we assess patient needs and direct patients into treatment. Currently, it takes two appointments and five to six hours for patients to receive a tentative treatment plan and fee estimate; our goal is to streamline the new-patient process so that this takes place in the first appointment. The task force is now beginning to formulate recommendations for implementing a clerkship system and is discussing an organizational structure for the new clinical model. Faculty and staff will be contacted for their suggestions as this process continues.

Project Management (led by Dr. John Wataha):

Meeting regularly since January, this task force has created a comprehensive inventory of the processes that make up the School of Dentistry. This inventory will guide us forward as we reorganize our curriculum, clinics and organizational infrastructure. The first phase of the inventory project will be completed in July, after which the task force will begin to formulate recommendations for a permanent Project Management Office. At the same time, it will continue to assist the other task forces as they analyze processes critical to our evolution toward still greater excellence at UWSOD.

Organizational Development (led by Dr. Rebecca Slayton):

This task force has distributed and collected data from all the academic units regarding names and titles of individuals in the unit, what duties they perform, how often and for how much time. This is intended to provide a snapshot of our School's current organizational state, to help us formulate a picture of the ideal state. The next steps will be to meet with individuals from each unit to clarify data and additional activities that may not have been captured in the matrix.

Currently I am serving on two of the task forces, namely, the Curriculum Working group and Clinical Systems. As one can perceive, the amount of work is daunting. However, those who are involved are all very excited and willing to contribute. Some of the details of the results will be published in a dental education journal.

OTHER ACIVITIES

In the spirit of involving all the communities within the institution, UWSoD has put together a series of activities, which include an Open Forum for all to ask questions, electronic updates from the Dean and Task Force leaders, and a biannual faculty retreat. As an example, the School also organized a full day faculty retreat in December 2012 and invited stakeholders, faculty /staff and students from UWSoD and from the University of Washington. Over 150 attendees gathered and discussed how the "Dentist of the Future" will be defined and the strategic implications for the UWSoD.

CONCLUSIONS

UWSoD has excellent clinical tradition and our students consistently perform very well in clinical board examinations with close to 100% pass rate. Although the School's patient-care services passed with flying colors during the last CODA accreditation with no suggestion and/or recommendation, we must continue to evolve as a dental school in order to thrive. The impending changes in dental and medical education gave us an opportunity to evaluate our current teaching models. The Dean has charged several Task Forces to revamp our systems. The School is undergoing some major changes with regards to clinical clerkship implementation and curriculum renovation.

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UWSoD Accreditation Report 2009

Science Session

Innovative Technologies for Biomolecular and Cellular Analysis

Mass spectrometry for high-throughput proteome analysis and biomarker discovery

Genomics Research Center, Academia Sinica, Taipei

C.H. Chen

Surface plasmon resonance for cell-based clinical diagnosis

Hiroshima University

M. Hide, Y. Yanase and T. Hiragun

Microarrays of plasmids and proteins for identifying the determinants of stem cell fates

Hiroshima University

K. Kato

On-chip cellomics technology for studying dynamics of cellular networks

Tokyo Medical and Dental University

K. Yasuda, F. Nomura, T. Hamada, H. Terazono and A. Hattori

Young Investigators' Session
—New Waves in BioDental Research from Hiroshima—

GCF and IFN-γin mouse periodontitis —Report of Brain-Circulation Program—

Hiroshima University

S. Matsuda

Effects of low-level laser irradiation on human dental pulp cell metabolism

Hiroshima University

R. Kunimatsu

Inhibition of cell-cell fusion during osteoclastogenesis by NHE10-specific monoclonal antibody

Hiroshima University

Y. Mine, S. Makihira and H. Nikawa

Generation of human induced pluripotent stem (iPS) cells in serum- and feeder-free defined culture from dental pulp cells

Hiroshima University Hospital

S. Yamasaki and T. Okamoto

Stem Cell Biology and Regenerative Medicine

Dynamics of Linage Fate Determination between Osteoblasts and Adipocytes in Rodent Models

Hiroshima University
Y. Yoshiko, K. Sakurai, Y. Fujino, T. Minamizaki, H. Yoshioka, Y. Takei, M. Okada and K. Kozai

Dental Pulp Cells as a Source for iPS Cell Banking

Gifu University
K. Tezuka

Linkage between muscle and bone

Kinki University
H. Kaji

Mass spectrometry for high-throughput proteome analysis and biomarker discovery

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Key words: proteomics, biomarker, mass spectrometry

ABSTRACT

Up to now, proteomics have not been extensively used in dentistry. Nevertheless, proteomic can possibly help dentistry in diagnosis and treatment evaluation. Proteomic studies of saliva, blood and tissue can be valuable to dentistry. In this work, we present the general approaches of proteomics, peptidomics and glycomics. Different biomarkers for different diseases and stem cells were identified. We expect the similar technologies can also be applied to dentistry.

INTRODUCTION

Both genomics and proteomics have been the root in large scale of identification of biochemical species. In the past, changes in protein abundance between samples can be quantitatively measured by the comparison of results from different spectra of 2-D gels. Nevertheless, reliable methods to determine amino acid sequence for proteins were not available until early 1990. Hillenkamp and his co-workers (Karas, 1988) developed matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) which can rapidly measure the molecular weights of different proteins with a time-of-flight (TOF) mass spectrometer. At about the same time, Fenn and his co-workers (Wong 1988) developed electrospray ionization (ESI) which can also give soft ionization of proteins. MALDI and ESI mass spectrometers became the two major ionization methods for protein analysis. In 1993, Henzel et al. (Henzel, 1993) reported the first work related to the identification of protein from the results of 2-D gel. The peptides were generated by *in-situ* tryptic digestion of proteins. Masses of different peptides were analyzed by MALDI time-of-flight (TOF) mass spectrometer (MS). The mass spectra patterns were used for comparison with known libraries to confirm peptides which can be further used for protein identification. In general, the mass resolution of a MALDI-TOF mass spectrometer is not high enough to give a non-ambiguous identification of a peptide with a high confidence. In addition, some amino acid residues have a very similar or even an identical mass. Collision-induced dissociation (CID) was used to obtain structure information with a tandem mass spectrometer. The first mass analyzer is used to determine the mass spectrum of the sample and the second mass spectrum (MS^2) for the structure of selected peaks from the first mass spectrum by a collision process of selected ions with selected gas molecules. Sometimes, higher orders of mass spectra (MS^n) due to CID can be obtained to get more information on the identification of biomolecular structures. In addition to CID, electron capture dissociation (ECD), infrared multiphoton dissociation (IRMPD), Electron transfer dissociation (ETD) were also developed to help on sequence determination.

Currently, there are two fundamental strategies for proteomics study. One is bottom-up and the other is top-down. In bottom-up approach, purified proteins or complex protein mixtures are subjected to chemical or enzymatic cleavage and the peptide products are usually separated by chromatography followed with mass spectrometry analysis. In top-down proteomics, intact protein ions or large protein fragments are subjected to gas-phase fragmentation for MS analysis directly. With top-down analysis, all post-translational modifications will be subjected to analysis while bottom-up analysis may skip the fragments with post-translation modifications. Since many fragmentation processes such as CID are not efficient for very large proteins (MW > 50000 Da), a true top-down strategy only works for relatively small proteins. In this work, most results are from bottom-up analysis for biomarker discovery.

It is well known proteins and peptides play critical roles in physiology. Biomarker searches have been pursued for various diseases including Alzheimer's disease, schizophrenia, depression and cancer. In this work, we use the peptidomic biomarker search to illustrate the peptidomics study for potential lung and gastric cancer diagnosis. Most proteomic/peptidomic samples were obtained either from tissue cells or body fluids. Proteins/peptides were extracted with C18 magnetic beads. For body fluid samples, they can be collected from blood, urine, sweat, saliva, gastric juice and etc. MALDI TOF/TOF mass spectrometer and LC-MS were used for peptidomic analysis.

There are two approaches to study proteomics. One is to do *de novo* amino acid sequencing to determine an entire protein in interest. The other is to determine a protein by some parts of amino acid sequence in a protein. Since there are often some "free" peptides in the cell or body fluids, it is important to separate "free" peptides

from proteins before a complete proteomic analysis. In the past, proteomic studies were usually pursued by 1-D or 2-D gel electrophoresis (2DE). Two-dimensional gel electrophoresis is still a very powerful method that resolves protein mixtures in the first dimension with isoelectric focusing. The second dimension has resolution based on molecular weight which is quite similar to mass spectrum except with lower mass resolution. Each visible band or spot in 2DE may represent one or a few proteins. In order to identify the proteins in the band, the gel can be cut out for enzymatic digestion followed with peptide mass spectrometry fingerprinting. Since 2DE is very time consuming, Ion exchange, HPLC and 2D HPLC are among the methods often employed to obtain good pre-separation for protein ID by mass spectrometry.

Up to now, most works on PTM are limited to phosphorylation and glycosylation. Phosphorylation is the addition of a phosphate (PO_4) group to a protein or peptide. Reversible phosphorylation of proteins is also a critical regulatory mechanism. Enzymes and receptors can be switched "on" and "off" by phosphorylation and dephosphorylation. For phosphorylation proteomics, total proteins were separated by SDS-PAGE. The gel was divided into several fragments, and then subjected in-gel digestion by trypsin. The tryptic phospho-peptides are subsequently purified separately by IMAC and TiO_2 magnetic bead. The identification of phospho-peptide sequences and phosphorylation sites can then be obtained by nano-HPLC and LTQ FT-MS analysis and followed by MASCOT search.

Glycosylation is the process of addition of saccharides to proteins. Two major types of glycosylation exist: *N*-linked glycosylation to the amide nitrogen of asparagine side chains and *O*-linked glycosylation to the hydroxy oxygen of serine and threonine side chains. The polysaccharide chains on glycoproteins serve important functions. For glycosylation analysis, biological protein samples preparation and separation are similar to the processes for phosphorylated protein analysis. The glycoproteome was analyzed by the following procedures: 1) the tryptic peptides were assayed by the similar procedures for proteomics; 2) the glycopeptides were purified by affinity magnetic bead and further processed for glycan analysis. First, the glycopeptides can be used for composition analysis by MALDI-TOF MS. Besides, the glycopeptides could be assayed directly by LTQ MS or LTQ FT-MS for the sequencing of polysaccharides. Second, the glycopeptides were subjected Peptide-*N*-glycanase F (PNGase F) reaction to release glycans and the glycan-free peptides were assayed by LTQ FT-MS for the identification of glycosylation site.

RESULTS

A. Peptidomic Analysis:

We use the peptidomic approach to do peptide biomarker search from gastric juice samples and exhaled air samples for gastric cancer and lung cancer diagnosis, respectively.

A-1. Gastric Cancer Analysis:

Gastric juice samples were collected by the typical endoscope method. After the insertion of the scope into the stomach, the gastric juice was aspirated through the suction of the endoscope and collected in a sterile trap. Gastric juice was then centrifuged for removal of contaminants after the sample collection. Then the samples were centrifuged. The supernatant was neutralized with ammonium hydroxide before purification by magnetic beads. The bound peptides/proteins were eluted using acetonitrile (ACN) after binding and washing. Reverse phase high-performance liquid chromatography (RP HPLC) was used to further separate proteins/peptides with an extend-C18 column. After the collections of the sample, a size exclusion chromatography was used to separate peptides from proteins. For peptidomic samples, a cut-off of 20,000 Da was often used (Chang, 2008). Five mass peaks correlated with stomach cancer were observed from experimental data. The mass to charge ratio (m/z) at 2187, 2387 and 3572 showed down-regulation but 2753 and 4132 showed up-regulation. The expression levels of these molecules are obviously different between cancer and non-cancer groups. Down-regulated peptides as pepsinogen fragments with the mass of 2187 Da and sequence of FLKKHNLNPARKYFPQW as well as 2387 Da with the sequence of FLKKHNLNPARKYFPQWEA were identified. Other down-regulated peptide with mass of 3572 Da was identified as a leucine zipper protein fragment with the sequence of ETKKTEDRFVPSSSKSEGKKSREQPSVLSRY. For up-regulated peptide with mass at 2753 Da in stomach cancer, it was identified as an albumin fragment with sequence of DAHKSEVAHRFKDLGEENFKALVL. For up-regulated peptide with 4132 Da, it was identified as a C-terminal fragment of α-1-antitrypsin with sequence of SIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK. According to the result, it suggests the existence of excessive amount of the above peptides related to albumin or α-1-antitrypsin in gastric juice can be served as a warning sign for a thorough check to prevent the progression to the stomach cancer. We found that the optimum combination for both sensitivity and specificity is when three of the peptides are used for cancer prediction. The overall sensitivity and specificity for gastric cancer were obtained as 79%, and 92% respectively.

A-2. Lung Cancer Biomarker Search from Exhaled Air:

Lung cancer is the most common cancer and the leading cause of cancer-related deaths worldwide. More than 80% of lung cancer cases belong to the non-small-cell lung cancer (NSCLC) subtype. There is an emergent need for valid diagnostic procedures aimed at screening lung cancer at an early stage. Breath analysis is one of the most desirable methods to identify new biomarkers for lung cancer. Exhaled breath condensate (EBC) can be collected by guiding and cooling exhaled air in a condenser system. EBC was used to measure peptides from exhaled air for lung cancer diagnosis (Chang, 2010).

The peptide constituents of EBC were purified by copper-coating magnetic beads and then subjected to the linear ion trap - Fourier transform ion cyclotron resonance mass spectrometer (LTQ-FTICR MS) analysis. Based on MS/MS analysis and MASCOT search, approximately 20 to 100 peptides in each EBC sample were identified. Dermcidin peptide E-R11 with the sequence of

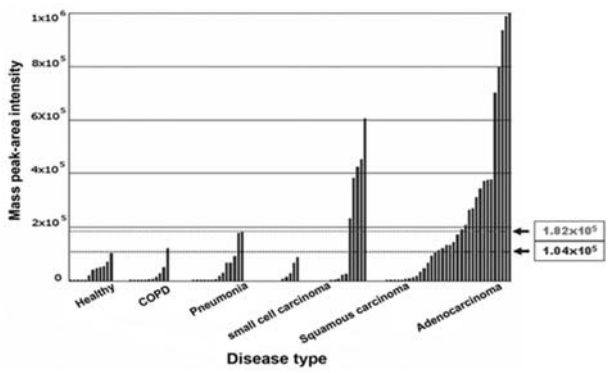

Fig. 1. Expression of E-R11 in EBC of healthy subjects and various lung disease patients

ENAGEDPGLAR was found to be more in EBC samples from NSCLC patients than those from normal control (Fig. 1). To characterize the biological activities of DCD in lung cancer cells, we determined the functional consequences of DCD expression knockdown effects by using lentiviral vector delivered shRNA. Real-time quantitative PCR was used to assay the knockdown effects of DCD. The results indicated that both cell lines H520 and PC13 can be infected at high efficiency by DCD shRNA lentiviruses and DCD shRNA can significantly knockdown the endogenous RNA expression of DCD in H520 (~70%) and PC13 (~80%). We further examined whether DCD expression knockdown results in growth reductions in normal or cancer cell lines. The results showed 20% and 30% growth reductions in H520 and PC13 cells respectively after DCD shRNA delivery, while there were no growth reductions in normal fibroblast and epithelial cells.

B. Proteomics:

We used proteomics to study liver cancer stem cells. CD133-positive liver cancer stem cells, which are characterized by their resistance to conventional chemotherapy and tumor initiation ability at limited dilution, have been recognized as a critical target in liver cancer therapeutics (Tsai, 2012). We investigated the proteome of CD133-positive liver cancer stem cells for the purpose of identifying unique biomarkers that can be utilized for targeting liver cancer stem cells. The subpopulation of hepatoma cells that expresses glycosylated CD133 antigen was sorted and compared with normal hepatocytes and CD133-negative hepatoma cells. Label-free protein quantitation was carried out by ID-based Elution time Alignment by Linear regression Quantitation (IDEAL-Q). The results have shown that 266 proteins were significantly regulated in CD133-positive hepatoma cells when compared with hepatocytes and CD133 negative cells. In order to search for unique surface markers, 617 membrane proteins were identified in CD133-positive hepatoma cells. Among these membrane proteins, we found two annexin family proteins (annexin 1 and annexin 3) that were either uniquely or highly expressed in CD133-positive hepatoma cells. Expression levels of CD133, annexin 1, annexin 3 and vimentin were further confirmed by RT-PCR, immunoblot, and immunocytochemistry analysis in CD133-positive and -negative hepatoma cells. These findings provide new insights in understanding the involvement of CD133 in hepatocellular carcinoma.

C. Glycoproteomic Analysis

Mutational activation of KRAS promotes various malignancies, including lung adenocarcinoma. Knowledge of the molecular targets mediating the downstream effects of activated KRAS is limited. We studied the KRAS target proteins and *N*-glycoproteins using human bronchial epithelial cells (HBECs) with and without the expression of activated KRAS ($KRAS^{V12}$). Using an OFFGEL peptide fractionation and hydrazide method combined with subsequent LTQ-Orbitrap analysis, we identified 5713 proteins and 608 *N*-glycosites on 317 proteins in HBECs (Reddy, 2012). Label-free quantitation of 3058 proteins and 297 *N*-glycoproteins revealed the differential regulation of 23 proteins and 14 *N*-glycoproteins caused by activated KRAS, including 84% novel ones. An informatics analysis prioritized some of the differentially regulated proteins (ALDH3A1, CA2, CTSD, DST, EPHA2, and VIM) and *N*-glycoproteins (ALCAM, ITGA3, and TIMP-1) as cancer biomarkers. A few validated proteins, including tumor suppressor PDCD4, were further confirmed as KRAS targets by shRNA-based knockdown experiments. Finally, *in vitro* invasion assay demonstrated distinct roles of TIMP-1 glycosylation in regulation of lung adenocarcinoma A549 and large cell carcinoma H1299 cell invasion. Together, our studies represent the largest proteome and *N*-glycoproteome datasets for HBECs.

DISCUSSION

It is highly desirable to be able to do single cell proteomics analysis. Recently, we developed a method which is able to trap and measure a single cell inside of an ion trap mass spectrometer (Chang, 2010; 2012). We further developed a biomolecular ion accelerator which can be used to measure a single molecular ion with mass-to-charge ratio (m/z) reaching to 30,000,000 (Hsu, 2012). We will try to combine this novel technology with proteomic analysis for top-down proteomic analysis (Fig. 2) in the future. A single cell will be trapped in the first ion trap. The trapped cell will be punched a hole by a laser beam. The released proteome will be sent to the second trap to obtain the mass spectrum. The identification can be obtained by the fragmentation of the collision between a selected protein and the target plate. The detection efficiency of very large biomolecular ions can be accelerated to enhance the detection efficiency. When the single cell

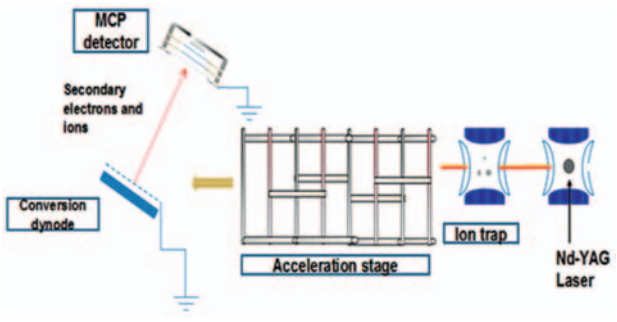

Fig. 2. Single cell proteomic device

proteomics can be achieved, it should have the possibility to lead to major breakthrough in biomedical research.

ACKNOWLEDGEMENT

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Surface plasmon resonance for cell-based clinical diagnosis

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Key words: surface plasmon resonance, imaging, cell, clinical diagnosis, function, allergy, cancer

ABSTRACT

Non-invasive real-time observations and the evaluation of living cell conditions and functions are increasing demands in life sciences. Most techniques for kinetic reactions and visualizations of cell activities require labeling of a key molecule to be studied. Surface plasmon resonance (SPR) has a great potential in that it reveals label-free, real-time binding kinetics of two biological molecules. We have reported that SPR sensors detect large changes of refractive index (RI) with living cells, such as mast cells, lymphocytes, and epidermal cells. The changes of RI by cell reactions largely reflect intracellular signal transductions, rather than the binding of stimuli to the cell surface. Based on these findings, we have developed a method to evaluate type I allergy using peripheral blood basophils with SPR. Moreover, we demonstrated that certain types of cancer cells may show irregular pattern of SPR signals in response to stimuli. To obtain a sensitive and high throughput system for SPR cell analysis, we developed a two dimensional SPR (SPRi) system that visualize single cell reactions. The establishment of rapid cell isolation technique suitable for SPRi should enable us to utilize SPR for new methods to evaluate cell functions and clinical diagnosis.

INTRODUCTION

Cell is the minimum unit of living creature from bacteria to vertebrates. Non-invasive real-time observations and the evaluation of living cell conditions and functions are increasing demands not only for basic research in life sciences, but also for various medical practices. Surface plasmon resonance (SPR) reflects reflective index in the field of evanescence on metal. The angle of resonance (AR) for SPR changes proportionally to the density of biological molecules in the evanescent field (ca. 200 nm) on the other surface of the metal, whose thickness is smaller than the wave length of the incident light. Thus, SPR can detect the association and dissociation of biological molecules on a surface gold film without any labeling in real-time manner. In the last two decades, SPR based-biosensors have been widely employed for label-free,

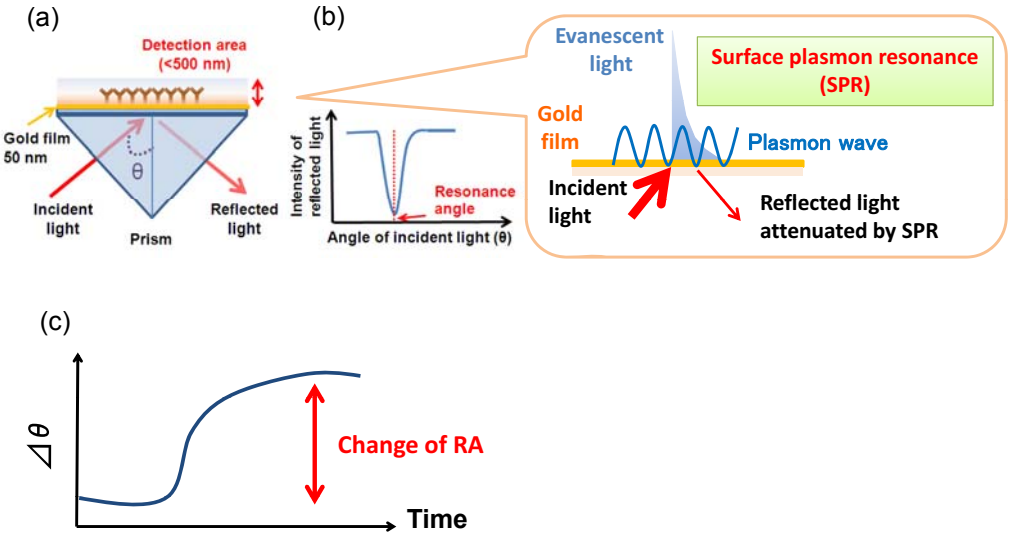

Fig. 1. Surface plasmon resonance (SPR) sensors detect a refractive index (RI) in the field of evanescence as an angle of resonance (AR).

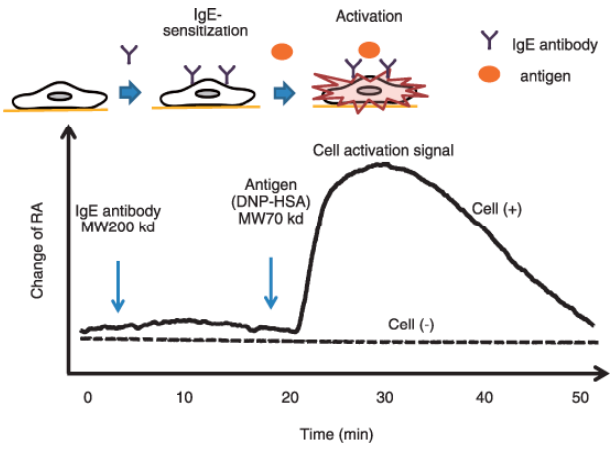

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Fig. 2. SPR signals (AR) obtained by the binding of anti-DNP-IgE and DNP-HSA to RBL-2H3 cells, which express the high affinity IgE receptor (FcεRI) on cell surface. Cells were cultured on the surface of the SPR sensor and incubated first with anti-DNP-IgE and then with DNP-HSA.

real-time analyses of two different biological molecules, such as antibody and antigen, receptor and ligand, and complimentary DNA fragments (fig. 1) in physiological conditions. In 2002, we first reported that living RBL-2H3 cells, a cognate rat mast cell line, caused an unexpectedly large increase of AR in response to biological stimuli beyond that due to a simple binding of IgE antibody to the cells (Fig. 2, Hide, 2003). Large changes of AR due to cell activations have been found in other cells, such as basophils and lymphocytes obtained from human blood, and epidermal cells (Yanase, 2011). To employ SPR for a real-time, label-free biosensor to study cell activities in a wide range of bioscience and clinical medicine, we studied the relation of SPR signals to intracellular signal transductions, and have developed a glass-fiber SPR that detect cell reactions on the fiber tip, and a 2-dimentional SPR system that visualizes single cell reactions.

CELL REACTIONS/MOLECULES DETECTED BY SPR

Since SPR sensors only detects changes of refractive

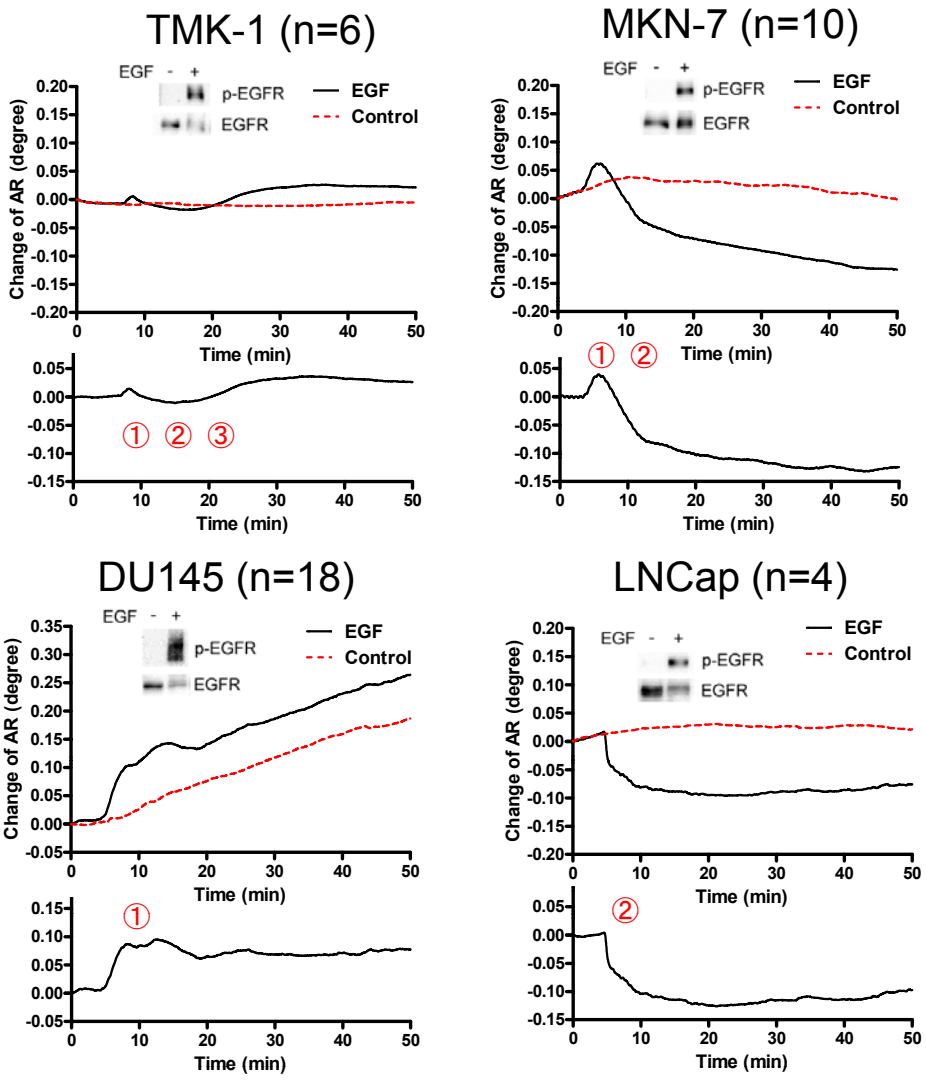

Fig. 3. SPR signals (AR) in four cell lines established from different cancers. TMK-1 cells showed week, but complete three phase changes of AR, whereas the other three cell lines showed unique incomplete patterns of SPR signals.

index (RI) in the evanescent field on the gold surface, subjects detected by an SPR sensor should be molecules in and around plasma membrane of the cells on a sensor chip. Thus, the increase of cell attachment should increases AR. Moreover, certain types of cells, including RBL-2H3 cells, and keratinocytes, increase attachment area in response to exogenous stimuli. However, the actual changes of AR by the activation these cells were much larger than the increase of cell attachment area. Furthermore, SPR signals (changes of AR) are tri-phasic, whereas the area of cell attachment of PAM-212 cells, a mouse keratinocyte line, simply increases during the measurment (Yanase, 2007). On the other hand, the abolishment of RBL-2H3 cell mobility by an actin polymerization inhibitor did not affect the SPR signal in response to antigen. Finally, the inhibition of receptor activities by molecular engineering has totally abolished SPR signals, preserving the binding activities of ligands (Hide, 2003; Yanase, 2007; Hiragun, 2012). These observations demonstrate that RI near plasma membrane of living cells dramatically changes in response to exogenous stimuli.

APPLICATION OF SPR FOR DIAGNOSOS OF TYPE I ALLERGY

The identification of causative antigens that is responsible for allergic symptoms in patients is crucial in the management of allergic diseases. Histamine release test using peripheral basophils *in vitro* is a safe and sensitive examination. In general, it is more reliable than the detection of antigen-specific IgE in serum. However, basophils of certain individuals do not release histamine, even if they are sensitized with IgE that binds to the antigen, due to dysfunctions in their intracellular signal transduction (non-responder). To overcome such a problem, we developed a method to detect SPR signals of peripheral blood basophils. Basophil-enriched leukocytes were purified and fixed on the surface of SPR sensor chip via monoclonal antibody against basophil surface antigen. When basophils sensitized with antigen-specific IgE were fixed on a sensor chip, they immediately caused an increase of RI in response to corresponding antigens, as did in response to anti-IgE, a positive control stimuli (Suzuki, 2008).

DIAGNOSIS OF CANCER BY SPR

The activation of epidermal growth factor (EGF) receptor (EGFR) on epidermal cells, such as keratinocytes, causes a unique triphasic change of AR, whereas the activation of other receptors, such as the high affinity IgE receptor (FcεRI) on mast cells and basophils, causes a monophasic increase of AR. Chinese hamster ovary (CHO) cells transfected with cDNA for EGFR showed a triphasic change of AR. However, when CHO cells were transfected with cDNA for EGFR containing a mutation at its kinase domain, they showed minimum change of AR. Moreover, a phosphatidylinositol 3-kinase inhibitor attenuated the third phase of AR change in CHO cells expressing wild-type EGFR. Furthermore, the pattern of AR change was independent on EGF concentration. These results suggest that EGF induces the SPR signals via the phosphorylation of EGFR, and that an impaired pattern of SPR signal induced by EGF may reveal a disorder in intracellular signal transductions of abnormal cells, such as cancer cells. In fact, we found that five out of six carcinoma cell lines show mono- or bi-phasic change of AR (Fig. 3). These results provide a possibility that the SPR biosensor could be applied to the real-time detection and/or diagnosis of malignant tumors.

OPTIC FIBER SPR

To apply SPR biosensors for the inside of the body,

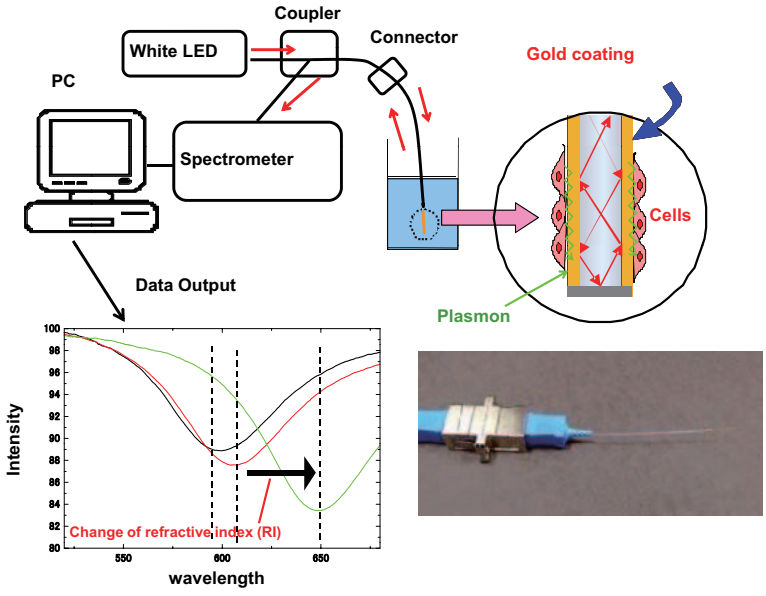

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Fig. 4. Construction of the optic fiber SPR. The core of 200 μm diameter with 1 cm length of an optical fiber was coated by gold film with 50 nm thickness. RBL-2H3 cells were cultured on the gold film and caused an increase of AR in response to antigen.

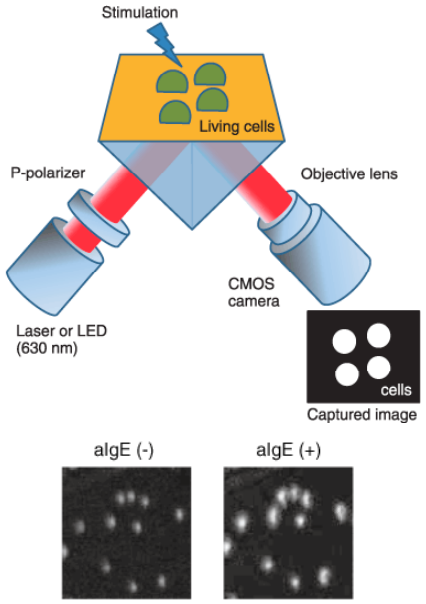

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Fig. 5. Structure of SPR imaging cell sensor and images of human basophils incubated with or without anti-IgE.

we developed an optic fiber SPR. The core of 200 μm diameter with 1cm length of an optical fiber was coated by gold film with 50 nm thickness. The light provided by a white LED and attenuated due to a SPR phenomenon in the sensor part was analyzed using a spectrum detector. The AR on a gold surface was indicated by the wavelength of the maximal absorption (Fig. 4). When RBL-2H3 mast cells were fixed onto the fiber tip, it detected a sustained increase of AR in response to antigen (Yanase, 2010).

SPR IMAGING FOR SINGLE CELL ANALYSYS

Although SPR sensor possesses great potential to reveal nano-scale living cell actions, conventional SPR sensors detect only an average of RI changes in the presence of thousands of cells. Moreover, it could provide only a small number of sensing channels (<10). Furthermore, they could not reveal the intracellular distribution of RI. We, therefore, developed a system of SPR imaging (SPRi) that determines a spatial RI distribution of individual cells. The sensor consists of a light source, CMOS detector, optical prism and a sensor chip with thin gold film matched to the prism via reflected index matching fluid (Fig. 5). Using this system, we detected reactions of individual rat mast (RBL-2H3) cells, mouse keratinocytes (PAM212 cells), human epidermal carcinoma (A431) cells, and human basophils (Fig. 5) in response to various stimuli, resembling signals obtained by conventional SPR sensor. Moreover, we could distinguish reactions of different type cells, co-cultured on a sensor chip. It is noteworthy that this system could detect reactions of basophils in response to various antigens in a very small drop of sample (0.7<μl).

DISCUSSION

The precise mechanism for cells to make such large changes of RI remained unclear. However, detections and/or analyses of cell functions by measuring RI have also been reported by other groups to date. Chabot et al, reported that SPR sensors detect real time adhesion and morphological changes in cells in response to various agents (Chabot, 2009). An SPR sensor based on Fourier Transform infrared FTIR-SPR operating in the near infrared wavelength range could monitor changes in cell occupancy and membrane biochemical composition, such as cholesterol (Yashusky, 2009; Ziblat, 2006). Lee et al, reported that an SPR sensor combined with olfactory receptor expressing cells provides a new olfactory biosensor system for detection of volatile compounds (Lee, 2009). Reactions of cancer cells against an anti-cancer drug with SPR sensor have also been reported (Kosaihira, 2008; Nishijima, 2010). In combination with a device to rapidly isolate basophils, lymphocytes, and/or tumor cells which may circulate in human blood, and a multi-well chamber, the SPRi technique should be a useful tool as a high throughput screening system not only for type I allergy, but also for various clinical diagnoses, such as drug hypersensitivity and cancer cell metastasis.

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CONFLICTS OF INTEREST

Authors declare no conflict of interest in relation to this study.

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Microarrays of plasmids and proteins for identifying the determinants of stem cell fates

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Key words: transfection microarray, protein microarray, stem cell differentiation, transcription factor, growth factor

ABSTRACT

Understanding the molecular mechanisms that underlie the dynamic behaviors of living cells is one of the most important challenges of current biology. To this end, various bioassay systems have been used to analyze the function of biomolecules in the context of diverse cellular events. This paper presents our attempts to develop new analytical platforms that permit the high-throughput functional analysis of multiple genes and proteins, especially with an aim to gain deeper insights into stem cell commitment. The platforms described here are characterized by the use of micro-processing technology to create cell-based microarrays.

INTRODUCTION

Networks of gene expression and protein interactions are key drivers for the phenotypic diversity of living organisms. In mammalian cells, thousands of genes are functionally connected each other, being involved in the regulation of dynamic systems. One of the important challenges of current biology is to elucidate molecular mechanisms that underlie the dynamic behaviors of living cells. Such information will further provide potential strategies to control cellular systems that is required especially for the rational manipulation of stem cell epigenetics and also for the tailored design of artificial matrices used in tissue engineering.

Conventionally, various bioassay platforms with cultured mammalian cells have been used to study gene functions. The key technologies include overexpression and knockdown of specific genes, taking advantage of plasmid vectors and small interfering RNAs (siRNAs). These methods are effective especially for annotating genes that code intracellular proteins such as trans-cription factors. In the case of proteins that exert their functions in extracellular spaces, such as growth factors, a simple addition of proteins to cells cultured *in vitro* allows us to gain information on their functions as was performed to date in numerous biological studies.

Because it is often that the number of genes or proteins of interests are quite large in the contemporary biological studies, we absolutely need to develop novel assay platforms that permit high-throughput functional screening of multiple genes. The present paper describes our attempts to establish new assay methods that utilize microarrays of nucleotides (plasmid DNAs and siRNAs) and proteins (extracellular matrices and growth factors). Two examples shown here demonstrate that our methods provide a large possibility to assess the role of genes and proteins and their interactions in the determination of stem cells fates.

TRANSFECTION MICROARRAYS

It is expected that functional genomics studies will provide rational strategies for manipulating cellular physiology and pathology through genetic intervention. One of the most important targets of such intervention may be facilitating the production of cells for regenerative medicine, as exemplified by the establishment of induced pluripotent stem cells (iPS cells; Takahashi & Yamanaka, 2006). An understanding of the networks of transcriptional regulation will aid efforts to regulate differentiation of precursor cells by means of direct genetic modification or treatment with extrinsic factors. These advances, however, require methodological innovation,

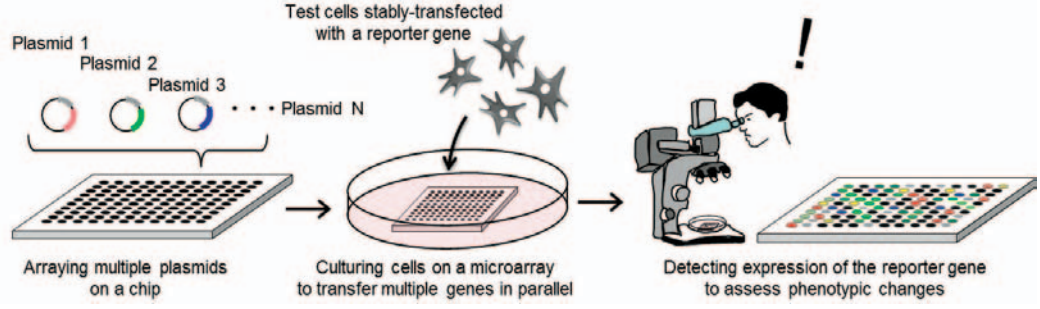

Fig. 1. Outline for the functional analysis of multiple genes using a transfection microarray

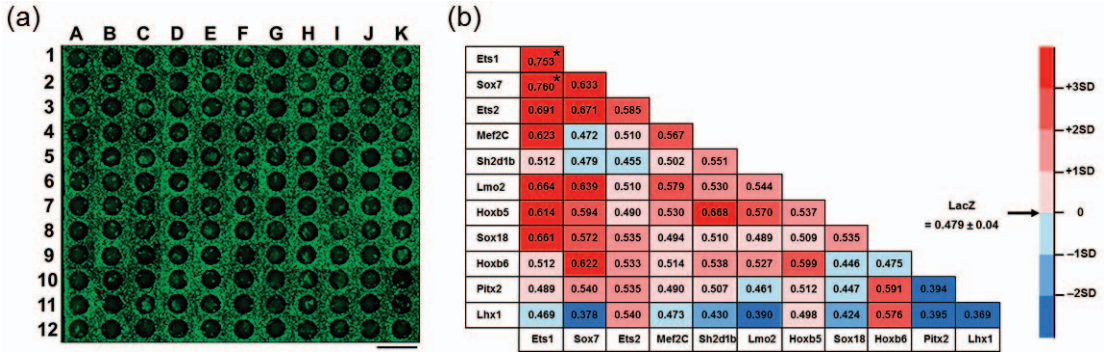

Fig. 2. EGFP expression of differentiated endothelial cells on the transfection microarray
(a) Low-magnification fluorescence image of EGFP-expressing cells after 4 days of culture of Flk1+ cells on the microarray. (b) Differentiation efficiency shown for all the genes and their combinations. Each box is colored depending on the difference in the efficiency compared to the control (LacZ).

so that large-scale functional assays can be conducted for thousands of genes in a high-throughput manner.

As shown in Fig. 1, transfection microarrays were prepared by microspotting multiple plasmid vectors together with a transfection enhancer onto a glass-based slide in an array format (Yamauchi, 2004). When seeded directly onto the array, cells take up the spotted plasmids and express the protein products. The principle of the microarray-based method is similar to that of expression cloning, however, the array format greatly enhances the throughput of analysis, because the method requires extremely small amounts of test vectors and, typically, 100 times less number of cells per gene.

To verify the feasibility of the microarray-based method, we applied the transfection array to identify transcription factors that affect vascular endothelial differentiation (Yamauchi, 2007). The transcriptional networks regulating vascular differentiation are not fully understood yet. We prepared expression plasmids for a panel of known factors (10 transcription factors and a signal transducer), and spotted them onto an array to effect transfection into vascular progenitor cells. The transcription factors we tested were selected according to large-scale expression profiles obtained by Gene Chip® analyses (Jakt, 2003), as well as published information with regard to vascular development (Yamashita, 2004). It is expected that such a focused microarray serves to significantly increase the hit rate of positive genes. Here we introduced plasmids into cells alone or in combination of two different genes.

Another important feature of this study is to use embryonic stem (ES) cells as a source of vascular progenitors. ES cells can proliferate *in vitro* in an undifferentiated state for a prolonged period while maintaining the ability to differentiate into all types of cells including endothelial cells. It was reported (Nishikawa, 1998) that lateral plate mesoderm could be induced from ES cells in an *in vitro* culture on type IV collagen-coated dishes. The differentiated cells were shown to express vascular endothelial growth factor receptor-2 (Flk1). Purified Flk1-expressing cells (Flk1+) were characterized as vascular progenitors that could reproducibly differentiate into endothelial, mural and blood cells under defined conditions (Yamashita, 2000). This *in vitro* system provides a platform useful for investigating cellular and molecular mechanisms underlying vascular development and therefore was utilized in this study for screening effective activators of endothelial induction.

The representative result is shown in Fig. 2 (Yamauchi, 2007). The results of screening successfully demonstrate that Ets1 and Ets1/Sox7 combination are the potent activators. Interestingly, other transcription factors, such as Lhx1 and Pitx2, were shown to inhibit endothelial differentiation. It is likely that the success of screening largely relies on the well-defined culture system for endothelial differentiation from ES cells, as well as the reporter system using the Flk1 p/e-EGFP construct. The selection of transcription factors also seems to be a major aspect that determines the hit rate of screening.

In the transfection microarray method described above, a panel of plasmids or siRNAs (Fujimoto, 2006) is arrayed on a small chip, and then cells are directly plated onto the array for transfection. In many cases, transfection is promoted by complexing nucleic acids with a cationic lipid enhancer. However, the efficiency of transfection is limited and, more importantly, transfection cannot be temporally controlled. Another advanced method that uses electric pulses to trigger the transfection of plasmids (Yamauchi, 2004) or siRNAs (Fujimoto, 2008) permits more efficient and temporally controlled transfection on a chip.

PROTEIN MICROARRAYS

Another important class of biomolecules that has impact on the stem cell fates includes ECMs and growth factors. These proteins extrinsically exert their functions through specific receptors such as tyrosine kinase receptors and cell adhesion molecules. Here we demonstrate that microarray-based analytical methods are also useful for studying the role of such proteins in the determination of stem cells fates.

Neural stem cells (NSCs) have been extensively studied for treating neurodegenerative diseases and traumatic injury of the spinal cord through cell transplantation. In these attempts, the fate of transplanted cells seems to have great impact on the outcome of the therapy. However, it is not straightforward with current technologies to direct survival, proliferation, migration, dif-

ferentiation, and integration of cells after transplantation. For controlling the behavior of trans- planted cells, several research groups including us have been involved in tissue engineering approaches where injectable gels are used as a carrier for neural cells. In most cases, cell adhesive peptides or growth factors have been incorporated into carrier materials.

The utilization of cell adhesive peptides and growth factors has been inspired by natural microenvironments in which the behaviors of neural cells are precisely controlled by ECMs and signaling factors. However, the diversity of these proteins makes it difficult to select the most appropriate component to be incorporated into carrier materials. To identify the best candidate, we implemented protein microarrays on which various extracellular matrices and growth factors were combinatorially displayed (Nakajima, 2007; Konagaya, 2011) (Fig. 3). NSCs were cultured on the microarray to screen various biomaterials without knowledge *a priori* on their functions.

In this paper, we simply focus on growth factors, employing a microarray displaying multiple growth factors including bFGF, EGF, IGF1, BDNF and CNTF. Among various growth factors, we tested these five factors because previous literatures reported responsiveness of neural cells to these factors. In addition, these factors transduce signaling through the distinct class of receptors, while some of the intracellular signaling cascades partially overlap each other.

Five growth factors were synthesized as fusion proteins with hexahistidine residues (His) and arrayed on a chip as a single component or the combination of two factors through chelate linkage with Ni^{2+} ions fixed on a chip. To achieve site-addressable presentation of these factors, we employed the photo-assisted patterning of an alkanethiol self-assembled monolayer (Kato, 2005). The His-mediated chelating method allows us to simply immobilize different growth factors on a single chip through the identical chemical reaction under mild conditions. NSCs obtained from the rat embryonic striatum were cultured directly on the microarray for parallel functional assays to study the effect of growth factors on the maintenance of NSCs and the promotion/inhibition of neuronal and glial specification.

As an example of the information obtained from the assays with growth factor microarrays, the results of differentiation assays are shown in Fig. 4a (Konagaya, 2011). Cells were cultured on the array in a medium containing fetal bovine serum and retinoic acid. Generally, serum and retinoic acid are known to promote differentiation of NSCs, giving rise to reduced expression of nestin, a marker for NSCs. After 5-day culture, cells were immunologically stained using antibodies to markers for differentiated neurons (β-tubulin III) and astrocytes (GFAP).

As is seen, the expression of β-tubulin III was elevated on the spot with BDNF-His or IGF1-His alone and as one of the two components, with little effects of co-presented partners. These results indicate that neuro- nal dif-

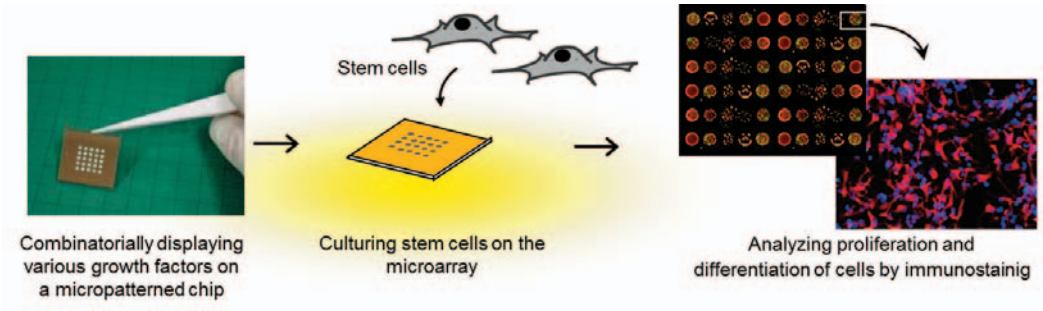

Fig. 3. Outline of the microarray-based assays for protein functions using stem cells

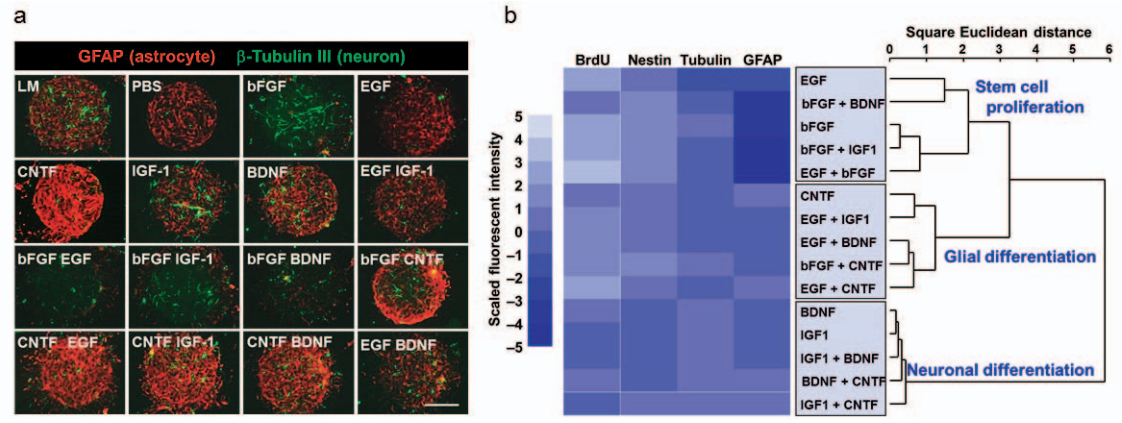

Fig. 4. The results of differentiation assays on the growth factor microarrays
(a) Fluorescent micrographs of cells stained using antibodies to β-tubulin III (green) and GFAP (red). Scale bar: 500 μm. (b) Dendrogram obtained from the hierarchal cluster analysis for 15 different growth factor conditions.

ferentiation was promoted on the spots with BDNF-His and IGF1-His. In addition, cells on the spots with bFGF-His alone or bFGF-His/CNTF-His in combi- nation abundantly expressed β-tubulin III.

With regard to the expression of an astroglial marker GFAP, it is obvious that cells on the spots with CNTF-His alone most abundantly expressed GFAP among the spots on the array, suggesting the enhanced differentiation of cells to glial lineage, especially to astrocytes. However, the GFAP expression was reduced depending on the partners co-presented with CNTF-His.

A hierarchal cluster analysis was performed for a data set obtained from immunofluorescent staining of BrdU, nestin (a marker for NSCs), β-tubulin III and GFAP for 15 different growth factor conditions. The resulting dendrogram is shown in Fig. 4b. It can be seen that 15 conditions are joined into three major clusters from A to C. Taking the immunostaining results into consideration, the cluster A appears to contain growth factor conditions that promote proliferation of NSCs, while the other conditions are grouped based on the similarity in their potential of inducing astrocytes and neurons in clusters B and C, respectively.

Immunostaining and cluster analysis revealed that bFGF-His and EGF-His as a single component promoted the proliferation of NSCs. In contrast, IGF1-His and BDNF-His promoted neuronal differentiation of NSCs, while CNTF-His did glial differentiation. These findings are overall in accordance with previous reports. The cluster analysis further provided new insights into the combinatorial effects of growth factors. It was found that the effects could be classified into four different traits: Combinations of two growth factors of different clusters are grouped in (i) the third cluster or (ii) the same cluster as either growth factor, but with reduced effects. Combinations of two growth factors of the same cluster are grouped in the identical cluster, with (iii) synergistic or (iv) destructive effects.

Accordingly, we gained deep insights into the effects of growth factors on the proliferation and differentiation of NSCs. The combinatorial effects of growth factors are rather complicated, but the findings obtained here will serve to rationally design carrier materials in which cellular microenvironments are fine-tuned by incorporating growth factors for achieving proper cell fates and functions. It appears that this complexity is attributed to the interference between partially-overlapping intracellular signaling cascades. Therefore, our results also provide a basis for deeper investigations on the crosstalk between growth factor receptors.

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On-chip cellomics technology for studying dynamics of cellular networks

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ABSTRACT

We have developed methods and systems of analyzing epigenetic information in cells, as well as that of genetic information, to expand our understanding of how living systems are determined. A system of analyzing epigenetic information was developed starting from the twin complementary viewpoints of cell regulation as an 'algebraic' system (emphasis on temporal aspects) and as a 'geometric' system (emphasis on spatial aspects). As an example of the 'geometric' system, we have developed an quasi-*in vivo* hiPS cardiomyocyte network assay and confirmed that it can predict the risk of lethal arrythmia correctly in 22 compounds. The knowlege acquired from this study may lead to the use of cells that fully control practical applications like cell-based drug screening and the regeneration of organs.

INTRODUCTION

Cells are minimum units determining their responses through genetic and epigenetic information like the history of interactions between them and fluctuations in environmental conditions affecting them. The cells in a group are also individual entities, and their differences arise even among cells with identical genetic information that have grown under the same conditions. These cells respond differently to perturbations. (Spudich & Koshland, 1976) Why and how do these differences arise? To understand the rules underlying possible differences occurring in cells, we need to develop methods of simultaneously evaluating both the genetic and epigenetic information not only for molecular level measurement but also for functional measurement. In other words, if we are to understand topics like variations in cells with the same genetic information, inheritance of non-genetic information between adjacent generations of cells, cellular adaptation processes caused by environmental change, the community effect of cells, we also need to analyze their epigenetic information. We thus started a series of studies to analyze epigenetic information among neighboring generation of cells and in the spatial structures of cell network to expand our understanding of how the fates of living systems are determined. As cells are minimum units reflecting epigenetic information, which is considered to map the history of a parallel-processing recurrent network of biochemical reactions, their behaviors cannot be explained by considering only conventional DNA information-processing events. The role of epigenetic information in the higher complexity of celluler groups, which complements their genetic information, is inferred by comparing predictions from genetic information with cell behaviour observed under conditions chosen to reveal adaptation processes and community effects. A system of analyzing epigenetic information should be developed starting from the twin complementary viewpoints of cell regulation as an 'algebraic' system (emphasis on temporal aspects; adaptation among generation) and as a 'geometric' system (emphasis on spatial aspects; spatial pattern-dependent community effect). The acquired knowlege should lead not only to understand the mechanism of the inheretable epigenetic memory but also to be able to control the epigenetic information by the designed sequence of the external stimulation.

As we can see in Fig. 1, the strategy behind our on-chip microfabrication method is constructive, involving three steps. First, we purify cells from tissue one by one in a nondestructive manner such like using ultrahigh-speed camera-based real time cell sorting, or digestible DNA-aptamer labeling. (Yasuda, 2000) We then cultivate and observe them under fully controlled conditions (*e.g.*, cell population, network patterns, or nutrient conditions) using an on-chip single-cell cultivation chip (Hattori, 2003; Inoue, 2001; Matsumura, 2003; Takahashi, 2003; Umehara, 2003; Wakamoto, 2001; Wakamoto, 2003) or an on-chip agarose microchamber system (Hattori, 2004; Kojima, 2005; Moriguchi, 2002; Suzuki, 2004a). Finally, we do single-cell-based genome/proteome analysis

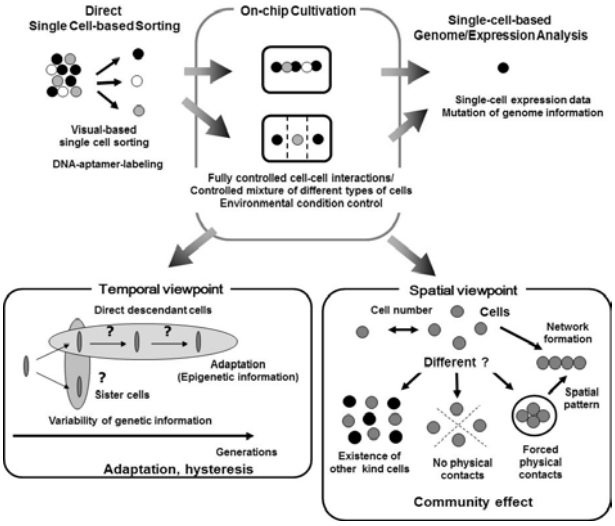

Figure 1. Our strategy: Three steps of on-chip single-cell-based constructive cellmoics analysis and the aim of this approach: temporal aspect and spatial aspect

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through photothermal denaturation and single-molecule level analysis (Yasuda, 2000).

In this paper, we explain the aims of our single-cell-based study using the on-chip single-cell-based cultivation/analysis system and introduce a part of the results focusing on the 'geometric' understanding of cellular systems using cardiomyocyte network as a practical example.

CULTIVATION SYSTEM FOR 'GEOMETRIC' VIEWPOINT: ON-CHIP AGAROSE MICROCHAMBER CULTIVATION

An approach to studying network patterns (or cell-cell interactions) and the community effect in cells was to create a fully controlled network by using cells on the chip (Fig. 1). For understanding the reaction of cells to the topography of the substratum, which occurs in the development and natural regeneration of tissue, a silicon wafer and a glass slide with holes and metal decorations have been created and tested (Brunette, 1983; Clark, 1987; Clark, 1991; Curtis & Wilkinson, 1997). Though these conventional microfabrication techniques provide structures with fine spatial resolution, it is still hard to change the shape of these structures during cell cultivation, which is usually unpredictable and is only defined during cultivation.

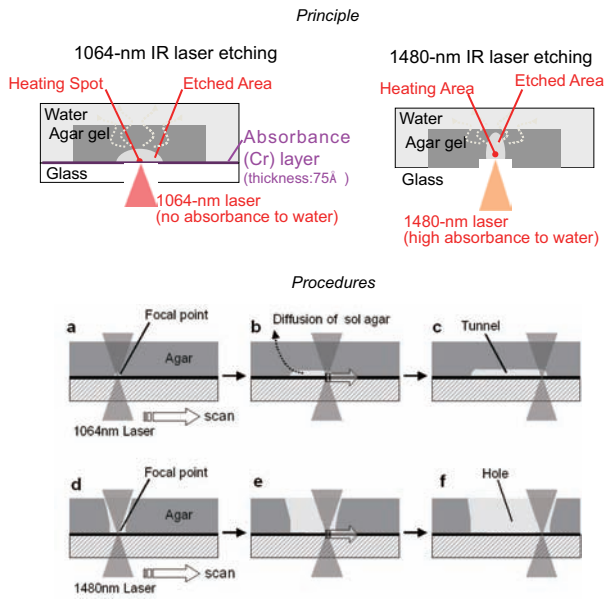

Figure 2. Schematic drawings of principles of 1064-nm infrared (IR) laser etching and 1480-nm IR laser etching, and their applications for microfabricatin formation
(a)-(c), Microtunnel formation using 1064 nm infrared focused laser beam, which does not have absorbance to water and agar; (d)-(f), microchamber formation using 1480 nm laser beam, which has absorbance to water and agar. The lasers melted the agar as follows: (a) When a 1064-nm infrared laser beam was focused on the chromium layer on the glass slide, the agar at the focal point near the chromium layer started to melt. (b) Then, when the focused beam was moved parallel to the chip surface, a portion of the agar at the heated spot melted and diffused into the water through the agar mesh. (c) After the heated spot had been moved, a tunnel was created at the bottom of the agar layer. (d) However, when a 1480-nm infrared laser beam was focused on the agar glass slide, the agar in the light path started to melt. (e) When the focused beam was moved parallel to the chip surface, a portion of the agar in the light path melted and diffused into the water. (f) Finally, after the heated spot had been moved, a hole was created on the glass slide.

We therefore developed a system consisting of an agar-microchamber (AMC) array chip, a cultivation dish with a nutrient-buffer-changing apparatus, a permeable cultivation container, and a phase-contrast/fluorescent optical microscope with a 1064-nm/1480-nm focused laser irradiation apparatus to create photothermal spot heating (Fig. 2) (Hattori, 2004; Kojima, 2005; Moriguchi, 2002; Suzuki, 2004b). The most important advantage of this system was that we could change the microstructures in the agar layer even during cultivation, which is impossible when conventional Si/glass-based microfabrication techniques and microprinting methods are used.

Significant advances have been made in developing analytical methods to monitor cell activity on a single cell level (fluorescence imaging, voltammetry, ion-selective electrodes, microelectrode arrays, combination of separation techniques with mass spectrometry). To meet the spatial resolution of those single cell level-monitoring technologies, micropatterning techniques for controlling of adequate spatial arrangements of cardimoyocytes, neurons and neurites have been developed and applied. While most of micropatterning techniques such as microcontact printing and microetching-based fabrication techniques are suitable for controlling the populations of dissociated cells with randomly arranged network patterns, those conventional micropatterning techniques can just control the orientation of spatial arrangements of their connections in pre-fabricated (ready-made) micropatterns, and, in principle, cannot control the directions of their elongation and connections. To overcome those problems, agar-microetching technique has been developed to fully control of spatial arrangements of single cells and the direction of their connectivity by flexible stepwise-fabrication of additional microstructures. This pioneering technique provides a constructive approach for spatial direction control and cell network formation during cultivation.

Agar microstructures can be photothermally etched by area-specific melting of agar microchambers by spot heating using a focused laser beam of 1480 nm (which is absorbed by water and agar gel), and of a thin layer made of a light-absorbing material such as chromium with a laser beam of 1064 nm (since water and agar itself have little absorbance at 1064 nm). For phase-contrast microscopy and μm-scale photo-thermal etching, three different wavelengths (visible light for observation, and 1480-nm/1064-nm infrared lasers for spot heating) were used simultaneously to observe the positions of the agar chip surface and to melt a portion of the agar in the area being heated. Using this non-contact etching, microstructures such as holes and tunnels can be created within a matter of minutes (Fig. 2).

QUASI-*IN VIVO* ASSAY FOR PREDICTIVE CARDIOTOXICITY

Lethal arrhythmia has been one of the major safety

concerns for the pharmaceutical industry in selecting and developing drug candidates. Integrated assay systems using hERG-transfected HEK-293/CHO-cells (hERG assay), isolated animal tissues (APD or MAPD assay) and conscious and/or anesthetized whole animals (QT or MAPD assay), are currently used to identify QT prolongation, whereas those assay systems are not competent to fully predict the potential lethal arrhythmia such as Torsades de Pointes (TdP) or ventricular fibrillation (Vf) induced by drugs or candidates. In this context, there is a longstanding and urgent need for a surrogate marker that can distinguish the torsadogenic potential from the QT interval duration. We have proposed a quasi-*in vivo* cardiotoxicity assay, which is a new *in-vitro* assay technology platform where human iPS/ES cell-derived cardiomyocytes and on-chip technology are combined and used as an assay tool to bridge the gap between pre-clinical studies and human clinical settings in terms of cardiotoxicity of new chemical entities for drug development (Fig. 3).

Potential advantages of the newly developed strategy of our quasi-*in vivo* assay include: 1) using a set of standard human cardiomyocytes prepared from human iPS/ES cells of different races, sexes and also from patients with various diseases to provide an ideal testing panel platform; 2) to predict lethal arrhythmia (TdP/VT/Vf) by evaluation of temporal fluctuation of single-cell-level ion channels kinetics, and by evaluation of spatial cell-to-cell conductance fluctuation using the on-chip cell network loop which can choose different conductance pathways of human cardiomyocytes among neighboring circulations; and 3) the capacity to quantitatively evaluate the correlation between calcium release and tension generation, and the inhibition on the trafficking pathway of ion-channel proteins by its long-term optical/electrical simultaneous measurement.

To study the re-entry cardiomyocyte cell network assay, we have developed the on-chip cell network cultivation system, and extra-cellular signals (field potentials: FP) of human embryonic cardiomyocytes in geometrically patterning chambers have been recorded with on-chip multi electrode array (MEA) system. Then, we have functionally reconstructed the normal and abnormal re-entry model of cardiomyocytes network loop from the viewpoint of propagation of contractile signals to be able to include the characteristics of heart into the chip like the functional spiral re-entry model (Figs. 3 and 4). And we found that the on-chip cardiomyocyte cell network assay is expected to be one of the candidates having the potential to measure the TdP and VF probability as pre-clinical testing for cardiac safety.

The data obtained in our laboratory indicates that the torsadogenic potential of 22 QT prolonging and non-QT prolonging drugs including false-negative/false-positive compounds in the current *in vitro* assays have been predicted correctly by quantitative evaluation of spatiotemporal fluctuation increase, and evaluation of hERG channel trafficking inhibition with longer exposure of compounds. Moreover, we have shown that the on-chip cell network loop model would offer the novel platform to assess the proarrhythmic (not only TdP but also VT/Vf) risks of compounds.

CONCLUSION

We have demonstrated the on-chip cell network assay, We developed and used a series of new methods of understanding the meaning of genetic and epigenetic information in a life system exploiting microstructures fabricated on a chip. The most important contribution of this study was to be able to reconstruct the concept of a cell regulatory network from the 'local' (molecules expressed at certain times and places) to the 'global' (the cell as a viable, functioning system). Knowledge of epigenetic information, which we can control and change during cell lives, complements the genetic variety, and these two kinds are indispensable for living organisms.

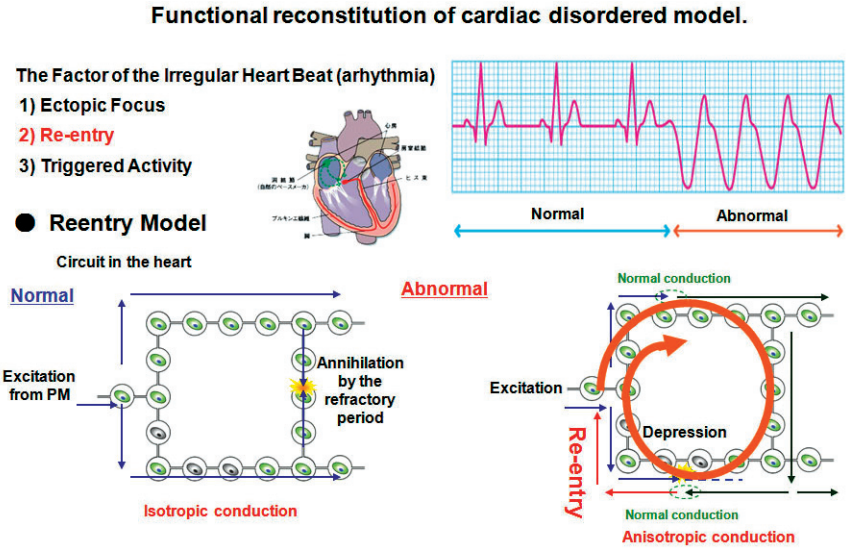

Figure 3. Concept of single-cell-based on-chip re-entry model as an example of quasi-*in vivo* assay for pre-clinical testing for TdP prediction
The network formation enables us to make a model of the signal propagation in the heart tissue.

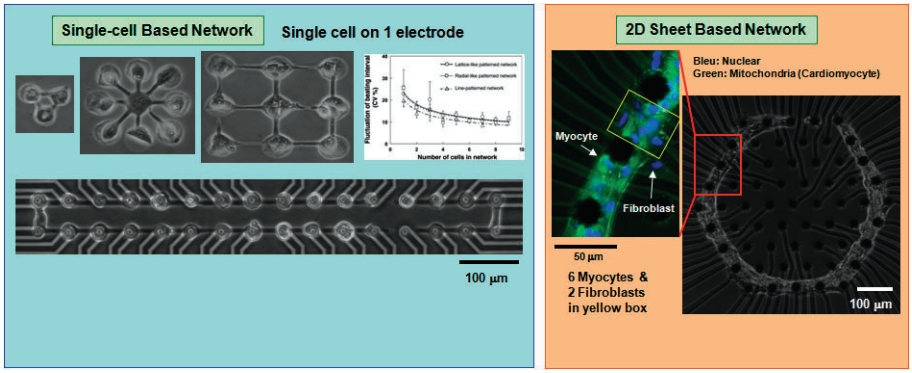

Figure 4. Formation of single-cell-based re-entry cell circuit in the agarose microstructures on a MEA chip

This new kind of knowlege has the potential to be the basis of cell-based biological and medical fields like those involving cell-based drug screening and the regeneration of organs from stem cells.

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GCF and IFN-γ in mouse periodontitis —Report of Brain-Circulation Program—

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Key words: Brain-Circulation Program, GCF, IFN-γ, periodontitis, bone resorption, pro-inflammatory cytokine

ABSTRACT

Program for Accelerating Brain Circulation performed by Japanese Society for the promotion of Science (Brain-circulation program), provided the opportunity for universities and other research institutions, along with the research strategy of the international research organization. This program sent abroad young researchers engaged in world-class international joint research, to challenge the various issues.

Using a mouse model of silk-ligature-induced periodontal disease (PD), we report a novel method of sampling mouse gingival crevicular fluid (GCF) in order to test potential bone loss biomarkers. To sample GCF, the original PD-induction ligature was removed, and a fresh GCF-sampling ligature was placed in the gingival crevice for ten minutes. The levels of IL-1β, TNF-α and IL-6 in GCF increased at 24 h after placement of PD-induction-ligature, but gradually decreased. While the level of IFN-γ in GCF was marginal at 24 h, it gradually increased up to Day-7, a pattern which uniquely paralleled progressive periodontal bone loss. Local injection of recombinant IFN-γ to the ligature-mounted site upregulated GCF RANKL accompanied by increased bone resorption. These results suggested that IFN-γ derived from GCF is a potential biomarker of periodontal bone destruction.

BACKGROUND

Periodontal diseases (PD) are inflammatory bone lytic diseases caused by microbial infection, indicating that several opportunistic pathogens act on host cells to produce inflammatory cytokines and enzymes that destroy periodontal soft tissue and alveolar bone. Importantly, several such host-destructive inflammatory factors, or biomarkers, can be identified in gingival crevicular fluid (GCF). Among some 90 components in GCF thus far evaluated, interleukin (IL)-1β, IL-6, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α appear to be signature biomarkers that reflect the level inflammation in the periodontal lesion. In the bone resorption lesion of periodontal disease, it was demonstrated that an osteoclastogenic cytokine, receptor activator of NF-κB ligand (RANKL), is produced by adaptive immune cells, including T and B cells. Since osteoclastogenic activity of RANKL is regulated by its soluble decoy receptor osteoprotegerin (OPG), measuring RANKL/OPG ratio can indicate osteoclastogenesis. However, the RANKL/OPG ratio in GCF may not be a suitable biomarker for ongoing bone resorption because it remains unchanged, even after successful nonsurgical periodontal treatment. Thus, for accurate diagnosis, it is desirable to identify biomarkers present in GCF that are suggestive of ongoing periodontal bone loss. This study aimed to establish a new method to detect GCF in a mouse model of ligature-induced periodontal disease and, as a result, identify potential biomarkers involved in the bone resorption process caused by pro-inflammatory effector T cells.

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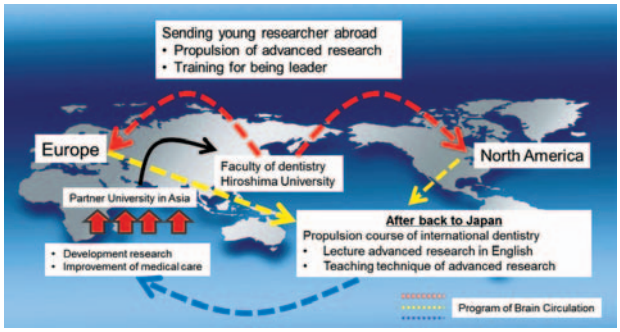

Fig. 1. The concept of Brain-circulation program promoted by Faculty of Dentistry in Hiroshima University
Young researchers who have been sent overseas from Hiroshima University Dentistry not only engage in cutting-edge collaborative research in overseas institutions, have experience the discussion research in the institution. After returning to Japan, young researchers will show the results in dentistry research course (study courses for students aiming to graduate school) in Hiroshima University, to participate in the operation of international dentistry courses. International dentistry course students (Southeast Asia, mainly foreign students from Indonesia, Vietnam, from Cambodia) brought back to home country this knowledge and concepts, try to bottom-up of dental medicine and medical care in your home country. Eventually it'll return as a raise of Hiroshima University School of Dentistry itself this achievement.

Effects of low-level laser irradiation on human dental pulp cell metabolism

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Key words: cell proliferation, cell differentiation, diode laser, pulp biology, tooth tissue engineering, regeneration

ABSTRACT

Laser irradiation is known to activate a range of cellular processes and can promote tissue repair. This study was aimed to examine the effects of the diode laser irradiation on the proliferation and osteogenic differentiation of the human dental pulp cells (hDPCs). The hDPCs culture was exposed to an 810-nm diode laser at a dose of 0 or 1.4 J/cm^2 and cell proliferation was evaluated. For osteigenic differentiation, the hDPCs at confluence were cultured in osteoblastic differentiation medium and irradiated daily with the diode laser. Mineralization and osteoblastic activity were evaluated by the alizarin red S staining and the measurement of alkaline phosphatase (ALP) activity and calcium concentration. The mRNA and protein expression levels of the bone/dentin markers were examined by qPCR and Western blot. Cell proliferation was significantly increased by the laser irradiation. Treatment with the laser also enhanced ALP activity and calcium concentration in the hDPCs culture, resulting in an upregulated mineralization. The mRNA expression levels of ALP, type I collagen (COL1) and dentin sialoprotein (DSP) in the laser irradiated groups were significantly higher than the non-treated controls. In conclusion, the 810-nm diode laser irradiation stimulates the proliferation and the differentiation of the hDPCs suggesting the possible contribution to the regeneration of the dental pulp.

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Inhibition of cell-cell fusion during osteoclastogenesis by NHE10-specific monoclonal antibody

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Key words: osteoclast, NHE10, cell-cell fusion, monoclonal antibody, bone resorption

INTRODUCTION

Alveolar bone is a key tissue to maintain oral functions. However, in inflammatory disease such as periodontitis and peri-implantitis, emergence of osteoclasts (OCs) is occurred. Bone resorption mediated by OCs is dependent on their differentiation from progenitor cells, which is elicited by the fundamental differentiation factors, macrophage-colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL).

The present study investigated i) mRNA expression patterns of the 10 NHE isoforms (NHE1–10) during osteoclastogenesis; ii) the effects of a monoclonal antibody (MAb) specific to mouse NHE10 on cell-cell fusion during sRANKL-dependent osteoclastogenesis iii) the effect of the 6B11 MAb on LPS-induced bone erosion *in vivo*.

MATERIAL AND METHODS

First, we prepared the rat NHE10 MAb. Rat MAb against mouse NHE10 was generated by immunizing Wistar rats three times with a mixture of synthesized peptides. Hybridomas were grown in protein-free hybridoma medium, and antibodies were purified with protein G-Sepharose (clones 6B11).

We used mouse RAW264.7 cell line and mouse bone marrow macrophages (BMMs) as *in vitro* osteoclast model.

RT-PCR, real-time quantitative RT-PCR, western blotting, confocal microscopy and immunoelectron microscopy methods were employed for analysis. In addition, osteoclast function was evaluated by TRAP staining, Pit assay, and RNA interference (RNAi).

Finally, to investigate the effect of the 6B11 MAb on bone erosion *in vivo*, mice were intraperitoneally injected with LPS in PBS or PBS alone in the presence of the 6B11 MAb or control IgG. We evaluated the femurs by micro-CT.

RESULTS

Among the 10 NHE genes investigated, time-dependent induction of mRNA expression in response to sRANKL stimulation was only detected for NHE10. Confocal and immunoelectron microscopic observation revealed that NHE10 protein was localized in plasma membrane.

A rat anti-mouse NHE10 MAb (clone 6B11) decreased the number of large TRAP-positive OCs, but increased the number of small TRAP-positive OCs. Similar results were obtained using siRNA silencing of NHE10 expression.

Representative images of micro-CT scans are shown. The ratio of bone volume to tissue volume (BV/TV) in the femurs of mice injected with LPS was significantly lower than that of control IgG or the 6B11 MAb alone. The decrease of the BV/TV ratio in mice injected with LPS was prevented by the 6B11 MAb.

CONCLUSION

In conclusion, the present study suggests that NHE10 may be a promising target molecule for the development of therapeutic modalities to prevent OC-dependent bone resorption occurring in osteolytic skeletal disorders.

Generation of human induced pluripotent stem (iPS) cells in serum- and feeder-free defined culture from dental pulp cells

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BACKGROUND

Human somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by introduction of transcription factors such as Oct3/4, Sox2, Klf4 and c-Myc. Human embryonic stem cells (hESCs) and human iPSCs (hiPSCs) can proliferate without limit and yet maintain the potential to generate derivatives of all three germ layers. These properties make them useful for understanding the basic biology of the human body, for drug discovery and testing, and for transplantation therapies. However, the original protocol for the derivation of hiPSCs required feeder cells and mouse embryonic fibroblasts (MEF) to provide a microenvironment for the reprogramming and the maintenance of human iPSCs. Moreover the inclusion of animal proteins makes those conditions complex and impractical for routine use, the variation in sources of those medium components is substantial. Therefore there exists batch variation in media components and it is unclear which factor (s) contribute to the cell growth and the maintenance of the undifferentiated cells.

OBJECTIVE

The purpose of this study was to generate and culture of human induced pluripotent stem cells (hiPSCs) in serum- and feeder-free defined culture conditions from dental pulp cells (DPCs) to elucidate the nature of the cytokine requirements of the cells to promote their self-renewal and inhibit their differentiation.

MATERIALS AND METHODS

Using a protocol approved by the Ethics Committee for Human Genome/Gene Analysis Research at Hiroshima University, we collected normal human third molars at Hiroshima University Hospital after having obtained informed consent for the usage of dental pulp cells (DPCs) to derive iPSCs. Primary human dental pulp cell cultures were established from discarded dental pulp tissues during surgery. These DPCs were reprogrammed into hiPSCs after transduction using *Oct3/4*, *Sox2*, *Klf4 and c-Myc* with retroviral vectors in serum-free medium.

RESULTS

We have established a fully defined serum-free culture system for the purposes of standardizing culture methods and protocols for deriving human iPS cells. Those generated hiPSCs can maintain proliferation, self-renewal and pluripotency. Furthermore, it has been confirmed that these cells could differentiate into cell types of the three germ layers by virtue of embryoid body formation in vitro and teratoma formation assay in vivo.

CONCLUSIONS

We have successfully generated hiPSCs from adult human dental pulp cells and maintained in an undifferentiated state in serum-free defined medium. As this simple serum-free adherent monoculture system will allow us to elucidate the cell responses to growth factors under defined conditions, and can eliminate the risk might be brought by undefined pathogens.

Dynamics of Linage Fate Determination between Osteoblasts and Adipocytes in Rodent Models

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ABSTRACT

Amongst mesenchymal lineages, osteoblasts and adipocytes share a common progenitor, and acquired conditions, including aging, regeneration and certain diseases, cause changes in fate allocation between the two lineages. During developmental progression at the single cell-derived colony level *in vitro*, osteoblast lineage cells were susceptible to an adipogenic fate switch via the activation of endogenous peroxisome proliferator-activated receptor (PPAR)γ, a master transcription factor of adipogenesis. Unexpectedly, a subset of relatively mature cell colonies exhibited osteo-adipogenic bipotentiality. Osteoblast lineage cells co-expressed non-osteoblastic lineage determinants, such as PPARγ, Sox9 and MyoD, amongst which only PPARγ was activated by rosiglitazone (RSG), a synthetic ligand of PPARγ. RSG increased marrow adiposity in parallel with bone loss in diet-induced obesity (DIO) mice. A direct tissue analysis using MALDI imaging mass spectrometry provided biomolecules involved in bone phenotypes in DIO mice treated with RSG. In an acquired mass spectrum, fibroblast growth factor (FGF)21, a hepatokine that regulates glucose and lipid metabolism, was detected. Therefore, a subset of osteoblast lineage cells have the potential to switch fate to adipocytes, which may be accelerated by the disruption of systemic and/or local regulatory mechanism(s).

INTRODUCTION

Multipotent mesenchymal stem cells differentiate into osteoblasts, adipocytes and other mesenchymal lineages, and key transcription factors underlie the commitment and fate choices of cells to particular lineages with the suppression of alternative lineages. Common disorders, such as osteoporosis, diabetes, obesity and chronic kidney disease, directly impinge on a reciprocal decrease of osteogenesis and increase of adipogenesis in the bone marrow in a peroxisome-proliferator activated receptor (PPAR)γ-dependent manner. Homozygous PPARγ-deficient embryonic stem cells fail to differentiate into adipocytes but spontaneously differentiate into osteoblasts (Kawaguchi, 2004), suggesting that fate allocation between the two lineages is determined during early development. However, the fate changes that occur at commitment or differentiation stage(s) remain uncertain.

PPARγ, a ligand-activated transcription factor belonging to the nuclear hormone receptor superfamily, is principally expressed in adipose tissue and heterodimerizes with a retinoid X receptor (RXR) to bind the PPAR response elements within promoters, including adipocyte-associated genes. Therefore, PPARγ (PPARγ2, in particular) acts as a master determinant of the adipocyte differentiation program. The thiazolidinedione (TZD) class of antidiabetic agents, such as rosiglitazone (RSG), is a frequently used synthetic ligand for PPARγ (Ferre, 2004). TZD stimulates adipogenesis and inhibits osteoblastogenesis (*e.g.*, Ali, 2005) via its up- and downregulation of PPARγ and runt-related transcription factor 2 (Runx2), a master determinant of osteoblastogenesis, respectively. However, the counterregulatory actions of these transcription factors remain controversial (Yu, 2012). For example, adipocyte-, but not myocyte-, associated genes are transcriptionally induced during osteoblastogenesis (Garcia, 2002). Dissecting when during osteoblast lineage progression cells are susceptible to fate switches may be complicated by the fact that, although osteoblast differentiation is well characterized (Aubin, 2001), phenotypic heterogeneity of osteoblast lineage cells is observed (Candeliere, 2001).

It is now generally accepted that both ligand-dependent and -independent activation of PPARγ mediate multiple actions, including adipogenesis. Dietary and endogenous fatty acids and the prostaglandin D2 series act as natural ligands for PPARγ. Cofators, such as the p160 family, form a complex with a heterodimer of PPARγ and RXR, resulting in the transcriptional activation of target genes (Powell, 2007). A SUMOylation of the PPARγ ligand-binding domain is also involved in the PPARγ-dependent transcriptional activity (Pascual, 2005; Mikkonen, 2013). Several endogenous factors, including fibroblast growth factor (FGF)21 (Wei, 2012), vascular endothelial growth factor (Liu, 2012), β-catenin (Rahman, 2012) and microRNAs (Li, 2013), have been established to possibly participate in some of these PPARγ pathways, resulting in significant impairment of osteogenesis and/or adipogenesis. Taken together, systemic vs. local approaches are needed to assess the precise mechanism(s) of cell fate determination of osteoblast and adipocyte lineages. This review paper will highlight some of our previous findings regarding a correlation

between bone loss and marrow adiposity using a global analysis of single cell-derived colonies (Hasegawa, 2009; Yoshiko, 2010; Minamizaki 2012) and the bone tissue of diet-induced obesity (DIO) mice employing imaging mass spectrometry (MS), allowing for the identification of a direct link between molecular information and the spatial distribution of analytes within a single tissue section.

SINGLE CELL COLONY ANALYSIS

The temporal sequence of osteoblast differentiation in rat bone marrow and fetal calvaria cell cultures highlights the concept that these models reflect a preponderance of multipotential progenitors and osteoprogenitors, respectively. In contrast to that observed in the bone marrow cells, RSG induced adipogenesis without decreasing osteoblastogenesis in the fetal calvaria cells. Likewise, the colony formation assays revealed that RSG increased both colony-forming unit (CFU) adipocyte (as determined using oil red O staining) and CFU-alkaline phosphatase (ALP, a marker of osteoblasts) colonies. To obtain mature cells from fetal calvaria cells, ALP positive (ALP^+) and negative selection were driven by magnetic cell sorting. We found that adipocytes formed diffusely in all fractions when treated with RSG, raising the possibility that adipocytes may arise from relatively mature osteoblast lineage cells.

To determine whether the adipogenic potential is restricted to a specific subset of osteoblast lineage cells, we used a combination of single-cell colony assays and replica plating (Fig. 1). Osteoblast lineage colonies were retrospectively identified using their corresponding replicas in ALP and von Kossa staining. Over 320 colonies were collected at multiple time points; a portion of the cells of each colony was replated at a high cell density in the presence of RSG, while the remainder was collected for total RNA preparation. Approximately 250 colonies were successfully adapted to subculture, and, of these, 189 were designated osteoblast lineages, as verified using replica dishes (Table 1). The definitive osteoblast lineage colonies subcultured and treated with RSG were classified as either ALP^+ or oil red O^+ (for adipogenesis), double positive or double negative. Notably, the colonies picked at an early time point and subcultured were primarily monopotential for an osteogenic fate, while a bipotential fate was observed in colonies selected at later time points. Therefore, some osteoblast lineage cells, including cells already partially differentiated/maturing, may be able to adopt an osteo-adipogenic fate.

To conduct global transcriptomic profiling of mesenchymal lineage markers at the single-cell colony level, we amplified cRNAs, followed by global qPCR analyses. Of the 189 osteoblast lineage colonies, 97 were evaluable for an analysis. Based on their osteoblast marker expression and the established osteoblast hierarchy (Aubin, 2001), the colonies were rearranged into order from immature to mature stages of osteoblast lineage progression. It is important to note that all colonies listed were committed to the osteoblast lineage, as evidenced by the *Runx2* gene expression and cytochemical staining in replica dishes, as described above. There was a clear developmental stage dependency in the frequency with which monopotential or bipotential colonies occurred (Fig. 2). Unexpectedly, we found that the osteoblast lineage colonies co-expressed multiple lineage markers. Amongst these, significant differences were observed in the PPARα and PPARγ mRNA levels in the colonies that gave rise to an osteogenic monopotential vs. either an adipogenic potential or osteo-adipogenic bipotential in the presence of RSG (Table 2).

By assessing single cell-derived colonies, we showed

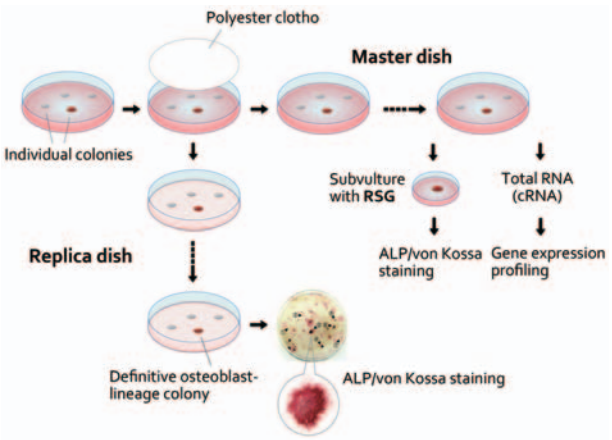

Figure 1. Replica plating of singe cell-derived colonies. Fetal rat calvaria cells were plated at limiting dilution in osteogenic medium. A few days later, polyester cloths were placed over developing colonies, then transferred upside down into new dishes with fresh osteogenic medium. Replica dishes were terminated at day 25 and subjected to ALP/von Kossa staining to confirm osteoblast colonies. Colonies in master dishes on multiple days were scraped and digested. The resulting cell suspension from each colony was split in half; one half was subjected to total RNA extraction and other half was subcultured in osteogenic medium with RSG. Osteoblasts and adipocytes were confirmed by ALP and oil red O staining, respectively.

Table 1. Summary of colony types and developmental fate of single cell-derived rat calvaria cell colonies.

Colony types (Days of cultures)	Number of colonies 12	15	17	21
Total, recovered from master dishes	106 (29)	73 (62)	80 (69)	81 (77)
Total, subcutured successfully	59 (25)	60 (49)	64 (54)	66 (64)
ALP positive	4 (3)	8 (8)	14 (14)	49 (49)
Oil red O positive	27 (18)	6 (5)	8 (7)	0 (0)
Double positive	3 (3)	28 (27)	21 (21)	5 (5)
Double negative	15 (1)	18 (9)	21 (12)	12 (10)

Number in parentheses indicate definitive osteoblast-lineage colonies retrospectively-identifed by replica plating.

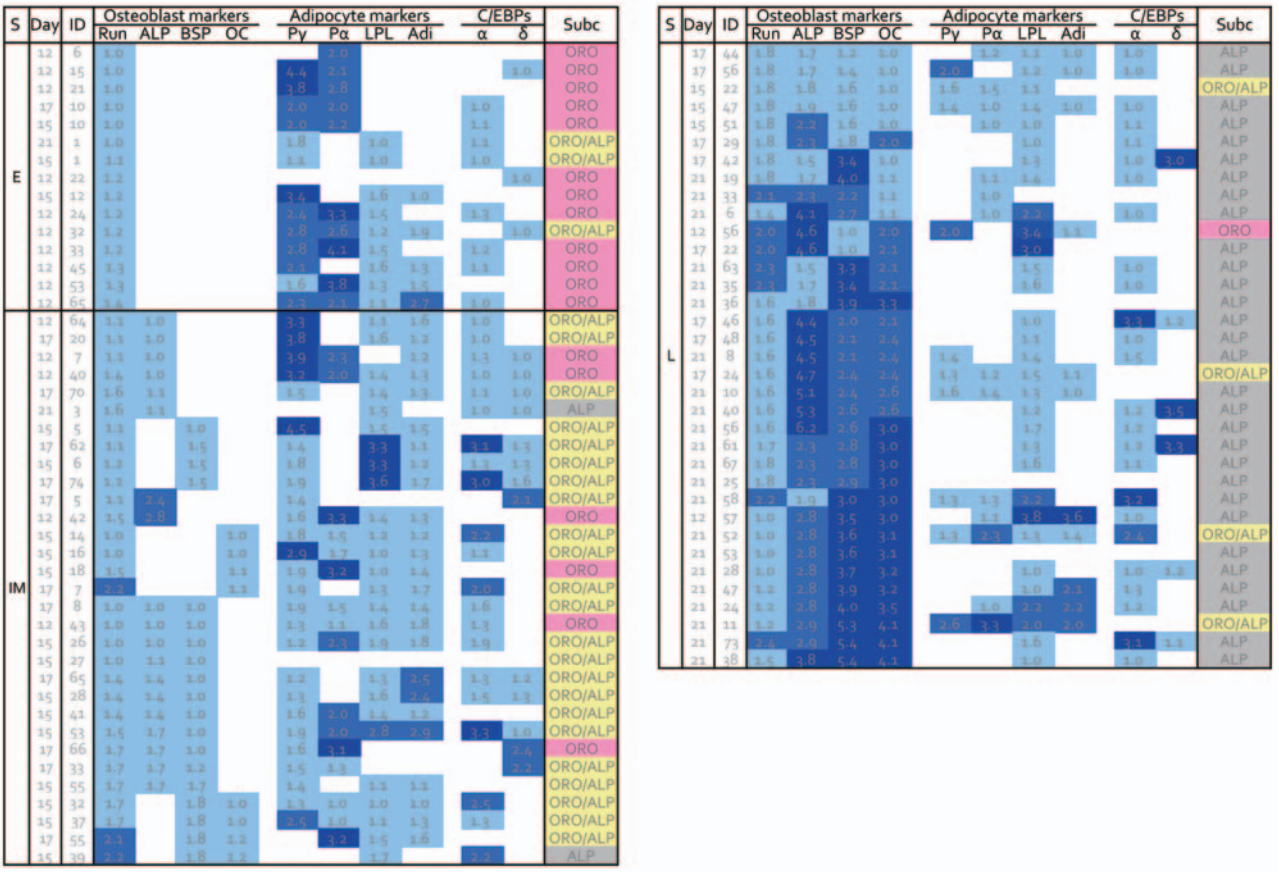

Figure 2. Gene expression profiling of osteoblast/adipocyte markers in single cell-derived rat osteoblast-lineage colonies and their osteo-adipogenic potential.
Numbers in each column denote relative mRNA levels of osteoblast- and adipocyte-related markers by qRT-PCR. Space, undetectable. The ribosomal protein L32 was used as internal control. S, Stages of osteoblast lineage progression, based on osteoblast marker expression; E, IM and L, early, intermediate and late stage, respectively. Day, Days of cultures; ID, colony ID. Run, Runx2; BSP, bone sialoprotein; OC, osteocalcin; Pγ and Pα, PPARγ and PPARα, respectively; LPL, lipoprotein lipase; Adi. Adipsin; C/EBPs, CCAAT-enhancer binding proteins. Subc, Subcultures; ORO, oil red O positive; ALP, ALP positive; ORO/ALP, double positive. Modified from *PLoS One* 5: e11782.

Table 2. Expression levels of PPAR and C/EBP mRNAs in osteoblast-lineage colonies correlates with their osteo-adipogenic potential when subcultured in the presence of RSG.

Number of colonies		19	30	32
Staining pattern in subcultures		Oil red O	Oil red O/ALP	ALP
Relative mRNA levels	PPARγ	2.22 ± 1.18*a*	1.82 ± 0.95*a*	0.24 ± 0.58
	PPARα	2.18 ± 1.22*a*	1.01 ± 1.22*bc*	0.35 ± 0.53
	C/EBPα	0.71 ± 0.78	1.12 ± 1.06	1.12 ± 0.84
	C/EBPδ	0.21 ± 0.42	0.47 ± 0.71	0.44 ± 0.98

aP<0.001 and *bP*<0.01, compared to matched ALP. *cP*<0.05, compared to matched. Modified from PLoS One 5: e11782.

that rat calvaria cells comprise a heterogeneous mixture of osteoblast lineage cells with different gene expression profiles and different potentials for fate switching. We then summarized the gene expression profiles of the mesenchymal lineage markers in a hierarchical assembly of single cell-derived osteoblast lineage colonies. Most lineage markers, including determinants for cells of a mesenchymal origin, such as Sox9 (chondrocytes) and MyoD (myoblasts), were highest early, while the levels of PPARγ_1 and γ_2 peaked somewhat later and subsequently decreased. These determinants were positive in the committed osteoblast lineages in the calvaria cell model. In the subcultures of the more mature cells (ALP$^+$ fraction), Runx2 was clearly located in the nucleus, while other determinants remained in the cytoplasmic (Fig. 3). The subcellular localization of Runx2 did not differ between cells treated with or without RSG, whereas PPARγ was primarily localized in the nucleus in the presence, but not absence, of RSG (Fig. 4). Consistent with this finding, RSG increased the levels of PPARγ and adipocyte markers; however, it did not alter the mRNA levels of the other determinants or osteoblast markers. These results suggest that a subset of osteoblast lineage cells express PPARγ that remains in the cytoplasm, while other cells

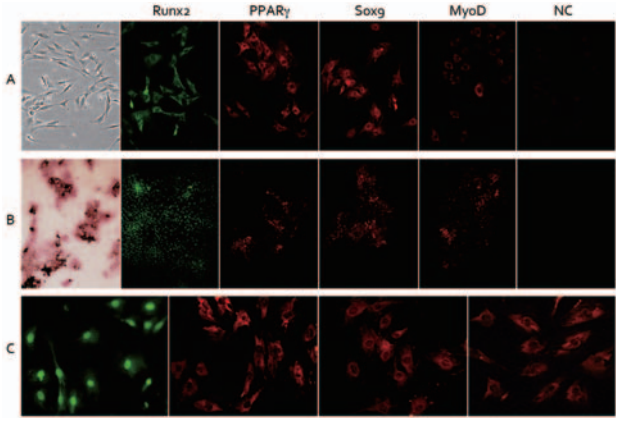

Figure 3. Subcellular distribution of mesenchymal lineage determinants in rat osteoblast-lineage cells.
Fetal rat calvaria cells were cultured in osteogenic medium with RSG. Proliferating cells (A), differentiating cells (B), and subcultures of ALP-positive differentiating cells (C) were subjected to immunofluorescence staining for Runx2, PPARγ, Sox9, and MyoD. Left-side panels of A and B show proliferating cells by phase-contrast microscopy and differentiating cells stained with ALP/von Kossa, respectively. Modified from *PLoS One* 5: e11782.

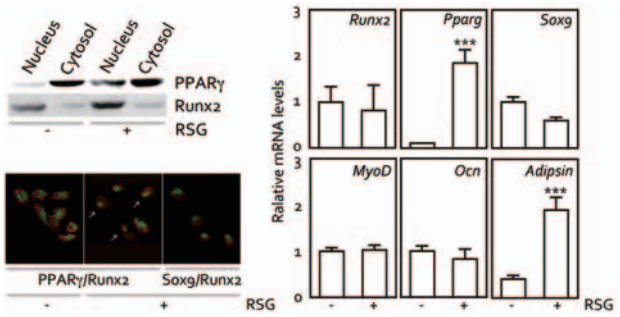

Figure 4. RSG promotes PPARγ actions in rat osteoblast-lineage cells.
ALP-positive fractions isolated from developing fetal rat calvaria cells were subcultured with or without RSG. (A) RSG increases relative abundance of PPARγ in the nucleus vs. the cytoplasm. The nuclear and cytosolic fractions were subjected to Western blotting for PPARγ and Runx2. (B) Double PPARγ and Runx2 nuclear positive cells are present in cells cultured with RSG. Cells were fixed and double stained with anti-Runx2 (green) and either anti-PPARγ or anti-Sox9 (red). (C) *Pparγ* and its target gene mRNA levels were increased by RSG. ***$P<0.001$, compared to vehicle control (–). Modified from *PLoS One* 5: e11782.

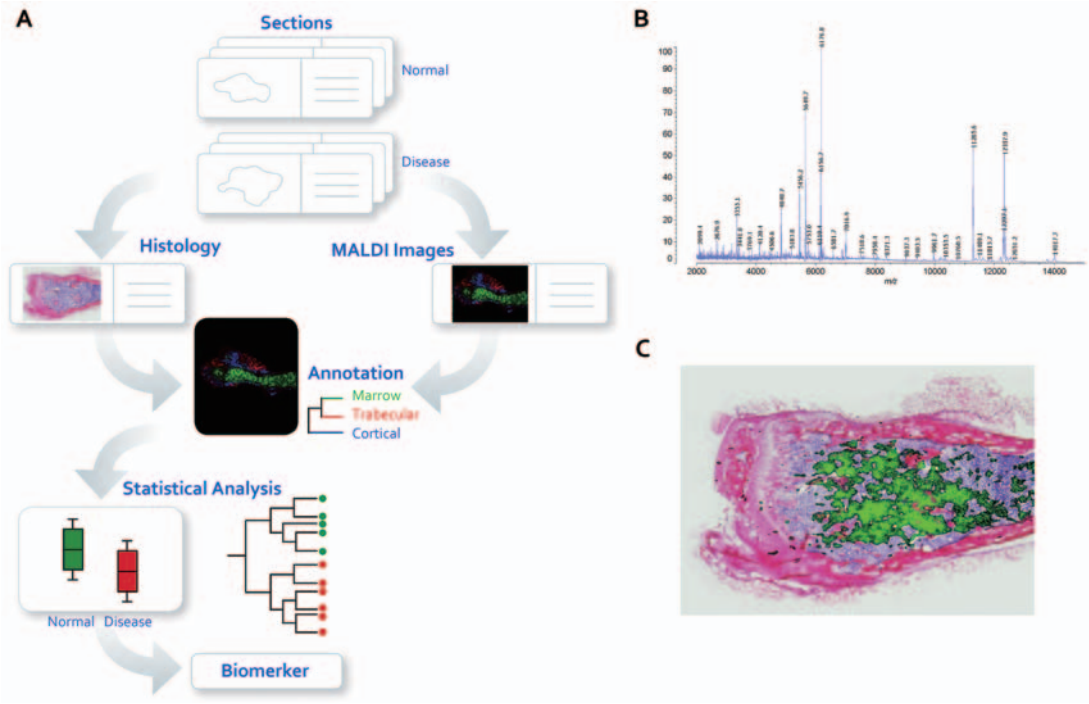

Figure 5. Direct Tissue-based protein analysis by imaging MS analysis. (A) Cryostat bone sections are covered with matrix with or without trypsin, of which a defined area is irradiated by a pulsed laser beam. The resulting desorbed ions are transferred to the mass spectrometer. Modified from an ultrafleXtreme Brochure (Bruker Daltonics). (B) Mass spectra from the proximal tibia. (C) The typical ion image merged with a tibial section stained with H&E. Modified from an ultraflex Xtreme Brochure.

can mobilize PPARγ to the nucleus; it is presumably these cells that convert into adipocytes under the stimulus of PPARγ-specific ligands.

MALDI MS IMAGING

Although *in vitro* and gene manipulation studies have contributed new findings regarding cellular and molecular events, the mineralized extracellular matrix always impedes analyses of biological processes in bone. Imaging MS is two-dimensional mass spectrometry used to visualize the spatial distribution of biomolecules that does not require either separation or purification of the

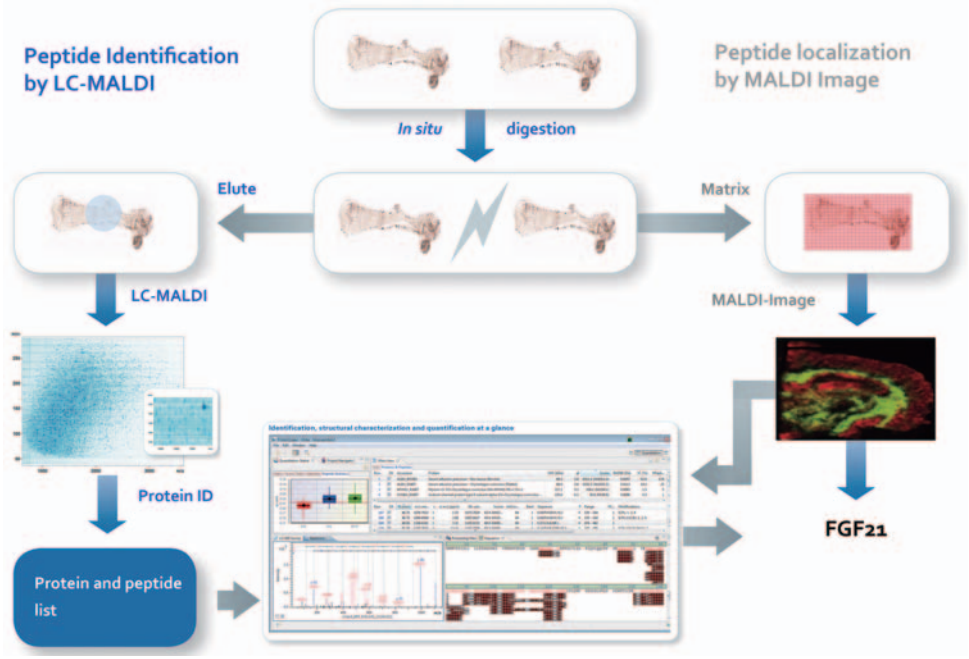

Figure 6. Protein identification workflow. Serial sections are sprayed with trypsin and matrix and subjected to either LC-MALDI MS/MS analysis or imaging MS analysis. The list of peptides acquired by database search and the ion image indicates FGF21 as a candidate for an adipogenic factor involved in DIO mice treated with RSG. Modified from an ultraflexXtreme Brochure.

target molecules (see for example, Liu, 2013). In general, thin cryosections are covered with a matrix and ionized using a pulse laser beam (matrix assisted laser desorption/ionization (MALDI)), followed by an analysis using a time-flight mass spectrometer (TOF-MS) at a resolution of 15 µm or more. This technology is still developing, although it is beginning to have an immeasurable impact on biophysics. We therefore used this method to identify biomolecules involved in osteo-adipogenesis in DIO mice treated with RSG.

Various challenges for imaging MS of proteins have been encountered in extraskeletal tissues (for example see, Liu, 2013) but not in bone. We began to establish sample preparation procedures suitable for imaging MS analyses of bone and then undertook to identify proteins uniquely distributed in bone sections obtained from DIO mice with or without RSG. Amongst the numerous signals observed in bone in both the normal and DIO mice at an average mass spectrum of 1,000-30,000 m/z, we demonstrated the signals in bone marrow (Fig. 5). It was found that an ion image of proteins at approx. 21,000 m/z was preferentially detected in the increased adipose tissue in the DIO mice with RSG. Using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of a tryptic peptide of extracted proteins, followed by the use of Mascot® search, we identified FGF21 as a candidate involved in the pathogenesis of DIO bone with RSG (Fig. 6). FGF21 is a hepatokine that beneficially affects carbohydrate and lipid metabolism (Kharitonenkov, 2005). β-Klotho, a single-pass transmembrane protein, converts canonical FGF receptors into specific receptors for FGF21 (Ogawa, 2007). Recent findings show that FGF21 promotes the SUMOylation of PPARγ in an autocrine manner (Dutchak, 2012) and that FGF21 gain of function and FGF21 loss of function lead to changes in fate allocation between osteoblast and adipocyte lineages by potentiating the PPARγ activity (Wei, 2012). Unfortunately, large proteins, including β-Klotho, are currently not available for imaging MS analyses, and hence we failed to detect β-Klotho in an imaging MS experiment.

We then used qRT-PCR to assess the possible role(s) of the FGF21-β-Klotho axis in bone. In normal mice, neither *Fgf21* nor *β-Klotho* was expressed in bone, while the expression of these factors was obvious in the white adipose tissue and liver. However, as expected, *β-Kltoho* mRNA was detected in bone marrow fractions in the DIO mice treated with RSG, suggesting that FGF21 cooperates with RSG to promote the PPARγ activity in DIO mice.

CONCLUDING REMARKS

We herein demonstrated that osteoblast lineage cells co-express Runx2 and either PPARγ, Sox 9 and MyoD or a combination of multiple mesenchymal lineage determinants and that, while Runx2 translocates to the nucleus during osteogenic differentiation, the latter do not, rendering them inactive under osteogenic differentiation conditions. However, the activation of PPARγ by treatment with RSG promotes the nuclear translocation of PPARγ and induces an adipogenic fate switch in a discrete subset of osteoblast lineage cells characterized by relatively high levels of endogenous PPARs. The molecular basis by which this subset of osteogenic cells acquires high expression levels of adipogenic transcription factors remains to be determined. To address this issue, we described the application of imaging MS technology to detect biomolecules in bone. Using this method, we identified FGF21 that may be involved in

RSG-induced adiposity and bone loss in DIO mice. Since FGF21 and its coactivator βKlotho appear to be expressed in cartilage and act in an autocrine manner (Wu, 2012), the role(s) of the FGF12-β-Klotho axis in fate allocation between osteoblast and adipocyte lineages in bone is under investigation in our lab.

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There is no conflict of interest associated with this study.

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Dental Pulp Cells as a Source for iPS Cell Banking

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Key words: dental pulp cell, iPS cell, HLA, cell bank, regenerative medicine

ABSTRACT

Human dental pulp cells (DPCs) are present in the cell population isolated from dental pulp tissues. We reported that viral introduction of four transcription factors (*OCT3/4*, *SOX2*, *KLF4*, and *c-MYC*) can reprogram DPCs into induced pluripotent stem (iPS) cells, which closely resemble embryonic stem cells. However, establishing quality-controlled iPS cell lines from a large number of individual patients is not easy and validation of them requires considerable time and cost.

Human leukocyte antigen (HLA) plays an important role in immune rejection of tissues and cells transplanted from allogenic donors. HLA haplotype-homo donors have a couple of identical HLA gene sets, resulting in presentation of HLA molecules half in the variation. Therefore, iPS cells derived from HLA haplotype-homo donors are expected to be successfully transplanted to a number of patients with less probability of immune rejection. We screened 177 DPC lines to find three patients having only one genotype in each of three HLA loci, A, B, DRB1. iPS cells established from these three patients were expected to show complete match with more than 23% of the Japanese population.

We believe that DPCs are one of the promising somatic cell sources for future iPS cell banking and regenerative medicine.

INTRODUCTION

Recent reports showed that human induced pluripotent stem cells (iPSCs) can be generated from several types of somatic cells with the defined transcription factors ; such as *OCT3/4*, *SOX2*, *KLF4* and *c-MYC* (Takahashi, Tanabe et al. 2007). Since reprogramming from differentiated state of the cells to pluripotency undergoes through a process of gradual change with morphology, gene expression patterns, and epigenetic state, it has been assumed that stem cells and progenitor cells are more amenable to reprogramming than that of differentiated cells. For example, Eminli et al. showed that neural stem and progenitor cells were reprogrammed more efficiently than other somatic cells do, suggesting that the differentiation state of the starting cell might affect reprogramming efficiency (Eminli, Utikal et al. 2008). However, generally undifferentiated stem cells are inaccessible (eg. neural stem cells in central nervous systems) or can be obtained as a very small fraction (eg. hematopoietic stem cells).

Human dental pulp cells (DPCs) can be obtained from both baby and permanent teeth in general dental therapy and contain a large number of multipotent stem cells (Gronthos, Mankani et al. 2000; Gronthos, Brahim et al. 2002; Stevens, Zuliani et al. 2008; Takeda, Tezuka et al. 2008; Zhang, Walboomers et al. 2008). In the previous study, we have established more than 150 DPC lines isolated from wisdom teeth of healthy volunteers and evaluated the potency of DPCs for iPS cell banking (Tamaoki, Takahashi et al. 2010). We randomly selected 6 lines to induce iPSCs and found that 5 DPC lines show higher reprogramming efficiency than that of human dermal fibroblasts (HDFs).

Cell transplantation therapy requires matching of human leukocyte antigens (HLAs), however, constructing a large cell bank that covers significant percentage of population is a hard task. Wisdom teeth are routinely removed in many clinics, thus allowing a broad spectrum of HLA types to be obtained. Considering this characteristic of DPCs, here we evaluated the potential of DPCs as a source of future iPS cell banks.

SOLUTION AND FUTURE PROSPECT

It should be quite useful for regenerative medicine to establish iPS cell banks with a sufficient repertoire of HLA types, since the establishment of clinical-grade iPS cell lines from individual patients would require significant time and cost. Therefore, one possible solution is conduction of cell transplantation without complete matching of HLA from allogenic donors. Then, in recent tissue transplantation therapy, immnosuppressants are frequently used to prevent immune reaction both host to graft and graft to host directions; however, when the immune system function is suppressed, there is an increased susceptibility to infectious diseases and cancers. Recently, Nakatsuji et al. estimated that a collection of 50 unique iPS cell lines having homozygous alleles of the 3 HLA loci (A, B, and DR) would cover ~90% of the Japanese population with a perfect match of these loci (Nakajima, Tokunaga et al. 2007; Nakatsuji, Nakajima et al. 2008). Then, we determined the HLA types of 177 DPC lines selected from our collecgtion, and found that 3 cell lines were homozygous for three HLA loci (Table 1). DP74, DP94, and DP263 had a couples of identical haplotypes whose genetic frequency in Japanese population are 8.735%, 1.458%, and 1.813, respecgtively; and, estimated to cover approximately 23% of the Japanese population with a perfect match (Table 2).

Table 1. DPC lines used for HLA typing.

Cell line	A-Locus		B-Locus		DRB1-Locus	
DP002	A2	A24	B7	B37	DR1	DR10
DP004	A3	A24	B7	B13	DR1	DR7
DP005	A2	A24	B60	B55	DR1	DR4
DP006	A2	A2	B35	B61	DR4	DR9
DP007	A3	A24	B44	B52	DR9	DR15
DP010	A24	A26	B13	B52	DR12	DR15
DP011	A2	A33	B13	B44	DR13	DR15
DP012	A24	A31	B7	B56	DR1	DR14
DP013	A31	A31	B61	B51	DR8	DR9
DP014	A24	A31	B62	B52	DR9	DR15
DP015	A2	A2	B75	B46	DR8	DR14
DP016	A2	A24	B7	B62	DR1	DR15
DP017	A24	A26	B52	B54	DR9	DR15
DP018	A2	A2	B35	B61	DR4	DR9
DP019	A24	A26	B62 (B15)	B61	DR4	DR9
DP025	A2	A24	B35	B51	DR4	DR8
DP026	A2	A2	B71	B35	DR4	DR12
DP028	A2	A31	B35	B46	DR4	DR8
DP030	A24	A31	B62	B52	DR9	DR15
DP031	A11	A31	B48	B55	DR9	DR11
DP032	A24	A26	B61	B61	DR4	DR8
DP035	A33	A33	B44	B44	DR8	DR13
DP038	A11	A31	B61	B51	DR4	DR14
DP039	A11	A24	B62 (B15)	B61	DR4	DR15
DP040	A24	A26	B62 (B15)	B52	DR14	DR15
DP041	A24	A26	B54	B54	DR1	DR4
DP042	A24	A24	B7	B51	DR1	DR8
DP044	A24	A24	B60	B52	DR12	DR15
DP046	A11	A24	B62 (B15)	B51	DR4	DR14
DP048	A24	A26	B62 (B15)	B52	DR14	DR15
DP049	A24	A33	B7	B44	DR1	DR8
DP052	A11	A26	B55	B67	DR8	DR16
DP053	A24	A31	B7	B54	DR1	DR4
DP054	A2	A26	B46	B61	DR8	DR9
DP056	A24	A24	B61	B52	DR9	DR15
DP057	A24	A33	B44	B54	DR4	DR13
DP060	A2	A24	B46	B52	DR8	DR15
DP062	A24	A24	B48	B59	DR4	DR9
DP064	A2	A2	B60	B46	DR8	DR14
DP065	A24	A24	B61 (B40)	B61 (B40)	DR9	DR14
DP066	A24	A24	B60	B51	DR8	DR11
DP068	A24	A33	B62	B55	DR4	DR4
DP069	A2	A24	B46	B52	DR9	DR15
DP072	A2	A11	B60	B46	DR8	DR15
DP073	A2	A31	B62 (B15)	B60	DR8	DR9
DP074*	A24	A24	B52	B52	DR15	DR15
DP075	A2	A24	B51	B62	DR8	DR8
DP080	A31	A33	B44	B51	DR9	DR13
DP081	A24	A24	B7	B52	DR1	DR15
DP083	A2	A26	B62	B60	DR8	DR15
DP086	A24	A31	B51	B52	DR9	DR15
DP087	A24	A33	B51	B52	DR14	DR15
DP092	A2	A24	B71	B35	DR4	DR15
DP094*	A11	A11	B62 (B15)	B62 (15)	DR4	DR4
DP095	A24	A24	B35	B61	DR4	DR9
DP096	A11	A24	B54	B58	DR8	DR13
DP097	A24	A24	B52	B54	DR4	DR15
DP099	A24	A31	B62 (B15)	B52	DR8	DR15
DP100	A2	A33	B35	B51	DR4	DR4
DP101	A33	A33	B44	B44	DR4	DR13
DP105	A2	A24	B35	B61	DR4	DR4
DP106	A24	A26	B62	B61	DR4	DR13
DP111	A33	A33	B44	B44	DR9	DR13
DP112	A26	A33	B44	B55	DR13	DR15
DP128	A24	A24	B62	B37	DR9	DR10
DP129	A2	A11	B39	B67	DR4	DR15
DP134	A31	A33	B44	B51	DR4	DR13
DP135	A33	A33	B62	B44	DR13	DR14
DP136	A11	A24	B52	B54	DR8	DR15
DP138	A2	A24	B61	B46	DR8	DR12
DP139	A2	A26	B62	B46	DR8	DR15
DP140	A31	A33	B62	B39	DR9	DR15
DP141	A2	A24	B60	B52	DR15	DR15
DP142	A2	A33	B62	B44	DR9	DR15
DP143	A24	A24	B54	B52	DR14	DR15
DP144	A2	A33	B44	B44	DR13	DR13
DP147	A24	A24	B51	B52	DR4	DR15
DP153	A2	A2	B62	B55	DR4	DR4
DP154	A2	A24	B62 (B15)	B52	DR15	DR15
DP156	A24	A26	B61	B61	DR4	DR8
DP157	A2	A33	B7	B44	DR1	DR4
DP158	A24	A33	B51	B52	DR9	DR15
DP159	A2	A24	B75	B46	DR8	DR15
DP160	A2	A24	B61	B54	DR4	DR13
DP163	A24	A26	B61	B46	DR4	DR9
DP164	A2	A24	B39	B60	DR12	DR12
DP165	A24	A24	B7	B62	DR1	DR9
DP166	A24	A33	B60	B44	DR4	DR16
DP167	A24	A24	B60	B52	DR4	DR15
DP169	A2	A26	B60	B51	DR8	DR15
DP170	A2	A24	B51	B54	DR4	DR9
DP172	A2	A24	B60	B52	DR4	DR15
DP173	A1	A33	B37	B44	DR10	DR14
DP174	A2	A24	B44	B48	DR4	DR13
DP175	A2	A33	B7	B44	DR1	DR4
DP176	A24	A24	B62	B61	DR4	DR9
DP177	A24	A33	B60	B44	DR13	DR14
DP178	A24	A24	B61	B52	DR12	DR15
DP179	A2	A24	B39	B51	DR8	DR15
DP181	A2	A26	B46	B48	DR8	DR9
DP182	A24	A31	B27	B59	DR13	DR15
DP184	A24	A33	B44	B52	DR13	DR15
DP185	A2	A24	B39	B51	DR15	DR15
DP186	A2	A11	B54	B54	DR4	DR14
DP187	A2	A2	B13	B46	DR8	DR12
DP193	A24	A26	B39	B52	DR8	DR15
DP191	A24	A26	B61	B61	DR9	DR12
DP194	A2	A2	B51	B51	DR12	DR14
DP192	A2	A24	B61	B52	DR4	DR15
DP195	A2	A11	B44	B46	DR12	DR13
DP196	A24	A24	B52	B55	DR9	DR15
DP203	A2	A24	B46	B52	DR8	DR15
DP202	A24	A31	B35	B52	DR10	DR15
DP198	A26	A31	B62	B51	DR4	DR9
DP204	A24	A33	B44	B59	DR4	DR8
DP206	A2	A24	B61	B59	DR4	DR9
DP205	A24	A26	B60	B51	DR4	DR14
DP207	A11	A31	B39	B51	DR4	DR8
DP209	A24	A26	B7	B39	DR1	DR8
DP210	A24	A24	B7	B62	DR1	DR14
DP211	A11	A33	B62	B44	DR4	DR14
DP212	A2	A24	B46	B52	DR9	DR15
DP213	A26	A33	B62	B44	DR4	DR13
DP214	A2	A31	B75	B61	DR9	DR9
DP215	A11	A24	B7	B67	DR1	DR16
DP217	A11	A24	B60	B46	DR4	DR8
DP219	A24	A33	B7	B44	DR1	DR9
DP218	A24	A33	B61	B44	DR8	DR13
DP220	A24	A26	B62	B35	DR4	DR9
DP221	A11	A26	B60	B54	DR14	DR15
DP224	A24	A24	B52	B54	DR14	DR15
DP222	A31	A33	B35	B44	DR4	DR14
DP226	A2	A26	B61	B61	DR9	DR14
DP227	A2	A2	B61 (B40)	B46	DR8	DR8
DP223	A24	A33	B61	B51	DR9	DR15
DP225	A31	A33	B7	B44	DR1	DR13
DP228	A24	A26	B71	B61	DR4	DR9
DP229	A11	A33	B60	B44	DR8	DR13
DP231	A2	A31	B46	B59	DR4	DR13
DP232	A2	A24	B60	B61	DR4	DR12
DP234	A2	A11	B39	B46	DR8	DR13
DP235	A33	A33	B7	B44	DR1	DR13
DP236	A2	A24	B44	B46	DR4	DR8
DP238	A24	A26	B39	B52	DR8	DR15
DP241	A2	A24	B46	B52	DR9	DR15
DP239	A2	A24	B7	B54	DR4	DR9
DP240	A24	A24	B62 (B15)	B61 (B40)	DR4	DR4
DP242	A2	A2	B13	B59	DR4	DR12
DP244	A24	A24	B61	B52	DR9	DR15
DP245	A3	A26	B61	B61	DR9	DR15
DP246	A2	A24	B51	B52	DR9	DR15
DP247	A2	A26	B35	B51	DR9	DR14
DP248	A2	A24	B44	B59	DR4	DR13
DP250	A24	A33	B40	B44	DR13	DR15
DP251	A24	A26	B7	B40	DR1	DR8
DP252	A24	A24	B7	B35	DR1	DR4
DP253	A24	A24	B52	B56	DR9	DR15
DP254	A2	A2	B48	B52	DR4	DR15
DP255	A26	A26	B35	B52	DR4	DR15
DP256	A24	A33	B44	B54	DR4	DR13
DP257	A24	A24	B40	B51	DR4	DR14
DP259	A24	A26	B35	B40	DR4	DR11
DP260	A24	A33	B52	B58	DR3	DR15
DP263*	A2	A2	B46	B46	DR8	DR8
DP264	A24	A33	B44	B59	DR14	DR8
DP265	A24	A24	B52	B52	DR8	DR15
DP266	A2	A26	B39	B40	DR4	DR15
DP268	A24	A33	B44	B52	DR13	DR15
DP269	A2	A31	B51	B59	DR4	DR15
DP270	A11	A24	B40	B40	DR12	DR14
DP271	A24	A33	B35	B44	DR9	DR13
DP272	A24	A31	B15	B40	DR9	DR11
DP273	A24	A26	B40	B48	DR12	DR15
DP274	A24	A33	B44	B59	DR4	DR10
DP275	A26	A33	B40	B44	DR9	DR13
DP276	A24	A24	B52	B54	DR4	DR15
DP277	A2	A11	B7	B51	DR9	DR12

HLA-A, B, and DRB1 loci were determined for 177 DPC lines.
*; Asterisks show 3-locus homo lines.

Table 2. HLA haplotype frequency in Japanese population.

Rank	Haplotype			HF*	Carrier	Accumulation	HHF**	N***
1	A*24	B*52	DRB1*15	8.735%	16.7%	16.7%	0.763%	131
2	A*33	B*44	DRB1*13	4.958%	9.7%	26.4%	0.246%	407
3	A*24	B*07	DRB1*01	3.763%	7.4%	33.8%	0.142%	706
4	A*24	B*54	DRB1*04	2.609%	5.1%	38.9%	0.068%	1469
5	A*02	B*46	DRB1*08	1.813%	3.6%	42.5%	0.033%	3042
6	A*11	B*15	DRB1*04	1.458%	2.9%	45.4%	0.021%	4704
7	A*24	B*59	DRB1*04	1.078%	2.1%	47.5%	0.012%	8605
8	A*24	B*40	DRB1*09	0.994%	2.0%	49.5%	0.010%	10121
9	A*11	B*54	DRB1*04	0.925%	1.8%	51.4%	0.009%	11687
10	A*26	B*40	DRB1*09	0.876%	1.7%	53.1%	0.008%	13031
11	A*24	B*51	DRB1*09	0.659%	1.3%	54.4%	0.004%	23027
12	A*24	B*46	DRB1*08	0.564%	1.1%	55.5%	0.003%	31437
13	A*31	B*51	DRB1*08	0.561%	1.1%	56.7%	0.003%	31774
14	A*24	B*40	DRB1*09	0.511%	1.0%	57.7%	0.003%	38296
15	A*26	B*40	DRB1*09	0.51%	1.0%	58.7%	0.003%	38447
16	A*02	B*40	DRB1*09	0.506%	1.0%	59.7%	0.003%	39057
17	A*02	B*35	DRB1*15	0.489%	1.0%	60.7%	0.002%	41820
18	A*02	B*13	DRB1*12	0.475%	0.9%	61.6%	0.002%	44321
19	A*02	B*39	DRB1*15	0.466	0.9%	62.6%	0.002%	46050
20	A*33	B*44	DRB1*08	0.452	0.9%	63.5%	0.002%	48947

*; HF represents haplotype frequency in Japanese population.
**; HHF represents haplotype homo frequency.
***; N indicates the size of screening needed to find a haplotype homo donor.

We have already established 2 HLA haplotype-homo iPS lines from DP74 and DP94 with safe method using episomal plasmid vectors (Okita, Matsumura et al. 2011). We calculated that these 2 lines would cover 16.6% and 3.0% of the Japanese population, respectively, based on the data disclosed by Central BoneMarrow Data Center of Japan Red Cross Society (http://www.bmdc.jrc.or.jp/stat.html). The easiness of isolation and handling of DPCs will make it easy to expand the size of the bank and even establish a stock of iPS cell lines homozygous for 3 HLA loci which covers nearly 50% of Japanese population by screening 10000 donors (Table 2).

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Linkage between muscle and bone

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Key words: bone, muscle, osteoporosis, sarcopenia, fibrodysplasia ossificance progressiva, osteoglycin

ABSTRACT

Sarcopenia as well as osteoporosis have been recently noted for rapid increase in the population of aged men and women. Numerous evidence suggest the existence of the interactions between muscle and bone. We investigated two aspects of muscle/bone relationships from clinical disease, such as fibrodysplasia ossificance progressiva, a disease linking muscle to bone. As a putative local inducer of muscle ossification, we found Tmem119, parathyroid hormone-responsive osteoblast differentiation factor. Moreover, osteoglycin might be one of muscle-derived humoral bone anabolic factor. The details in the links of muscle to bone remain unknown at the present time. However, this field may be important for the development of novel drugs for osteoporosis and sarcopenia.

INTRODUCTION

The research about the relationships between bone and other organ systems has been noticed recently. For example, the linkage between bone and cardiovascular system has been proposed from the correlations between osteoporosis and cardiovascular disease. Moreover, bone and glucose/lipid metabolism are mutually affected, and nervous system closely controls bone. Since muscle tissues are greatly affected by aging, sarcopenia has been clinically noted recently.

SARCOPENIA

Sarcopenia and osteoporosis are clinically important, as both are common in older people and increase in prevalence with aging. More than 30 % in older persons over the age of 80 years have sarcopenia. Locomotive syndrome are related to several age-related skeletal disorders, such as osteopososis, osteoarthrosis, spinal canal stenosis as well as sarcopenia. Sarcopenia is characterized by a deficiency in muscle mass. The European Working Group on Sarcopenia in Older People (EWGSOP) defines sarcopenia as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes (Cooper, 2012), which might lead to functional disability, a decrease in quality of life and an increased risk of death. Muscle mass is evaluated by appendicular skeletal mass index from dual-energy X-ray absorptiometry as well as bioimpedance analysis. Muscle strength is evaluated by the measurement of grip strength. Motor functional ability is evaluated by the measurement of gait speed. Diagnosis algorism for sarcopenia is proposed based on these parameters.

CLINICAL EVIDENCE ABOUT LINKAGE BETWEEN MUSCLE AND BONE

Numerous studies indicate that higher lean body mass (namely muscle mass) is related to increased bone mineral density (BMD) and reduced fracture risk, especially in postmenopausal women (Kaji, 2013). We previously reported that lean body mass is positively related to BMD in postmenopausal women (Nakaoka, 2001). Moreover, grip strength was positively related to forearm BMD and cortical bone thickness as well as bone strength index measured by peripheral quantitative computed tomograpgy (qCT) (Kaji, 2005). However, muscle parameters explained only less than 10 % of the variability of bone parameters (Jhannesdottir, 2012), suggesting that bone and muscle loss proceed at different rates with aging. Sclerostin, produced in osteocytes, suppresses bone formation by inhibiting canonical Wnt-β-catein pathway, an important bone formation signal. Recent study indicates that the increase in sclerostin levels with weight loss is prevented by exercise in obese older adults, and inverse relationship was found between the changes in sclerostin and lean body mass (Amamento-Villareal, 2012). Sclerostin may be responsible for exercise-induced changes of muscle and bone.

ENDORINE FACTORS AND MUSCLE/BONE RELATIONSHIP

Several endocrine factors simultaneously affect muscle and bone (Figure 1). Recently, the significance of vitamin D insufficiency in fracture risk and falling presumably related to muscle function has been recognized. Vitamin D has various actions on both muscle and bone cells, and vitamin D deficiency induces muscle atrophy, predominantly at type II muscle fibers. Our previous study indicate that endogenous growth hormone excess increases and decreases muscle mass and fat mass, respectively, as well as increases bone mass in acromegalic patients (Kaji, 2001). Thus, growth hormone posi-

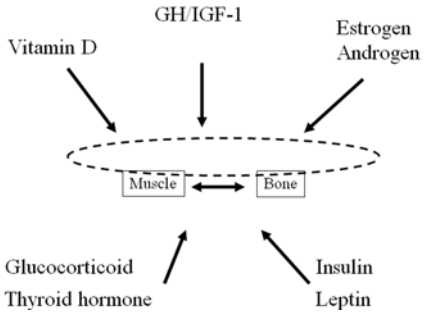

Figure 1. Endocrine factors influence muscle /bone relationship.
Vitamin D, growth hormone (GH), sex steroids, glucocorticoids, thyroid hormone, insulin and leptin affect both muscle and bone simultaneously.

tively affects both muscle and bone. Sex steroids, glucocorticoids, thyroid hormone, insulin and leptin also affect both muscle and bone. Genetic studies suggest that genetic factors explain 60-70 % of osteoporosis and sarcopenia. Several gene polymorphisms, such as androgen receptor, estrogen receptor, vitamin D receptor, insulin-like growth factor-I (IGF-I), low-density lipoprotein-related protein-5 (LRP-5), affect both osteoporosis and sarcopenia (Karasik & Kiel, 2008).

INTERACTIONS BETWEEN MUSCLE TISSUES AND BONE

As described, several clinical evidence suggest the interactions between muscle and bone. Moreover, fractures that are covered with relatively intact muscle improve rapidly than fractures associated with more severe damage. Muscle flaps applied to autogenous bone grafts improve healing. Proinflammatory cytokines at the site of fracture were found to induce the differentiation of stromal cells present in muscle into osteoprogenitor cells and were found to promote bone fracture healing. In the previous study, muscle-derived mesenchymal cells were more effective as the source of cells that differentiate into osteoblastic cells than bone marrow mesenchymal cells (Glass, 2011). These findings suggest that muscle tissues play some important physiological and pathological roles through certain interactions between muscle tissues and bone metabolism.

Fibrodysplasia ossificans progressiva (FOP) is a genetic disorder with progressive extraskeletal ossification, especially in muscle. FOP could provide important clues as a disease connecting muscle to bone. A heterozygous constitutively active mutation (R206H) in a bone morphogenetic protein (BMP) type I receptor, activin-like kinase 2 (ALK2), is responsible for the molecular pathogenesis of FOP.

MUSCLE-DEERIVED LOCAL FACTORS

We raised the hypothesis that there might be some local factors that enhance or suppress ossification specifically in muscle tissues. We therefore performed a comprehensive DNA microarray analysis between control and ALK2(R206H) stably transfected mouse myoblastic C2C12 cells (Tanaka, 2012a). In that study, we found that Tmem119 promotes the differentiation of myoblasts into

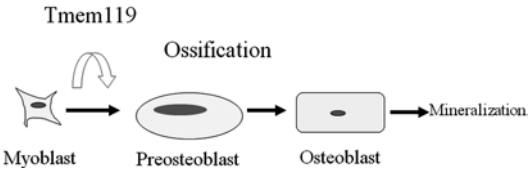

Figure 2. Local regulator for muscle ossifiaction
Tmem119 induces the commitment of myoblasts into osteoblasts.

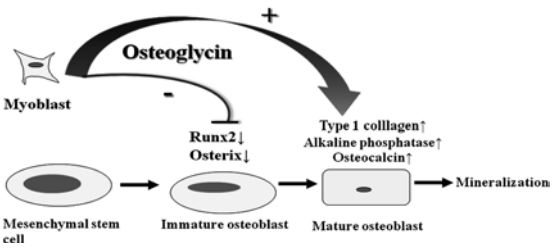

Figure 3. Role of osteoglycin in bone
Osteoglycin, produced from muscle tissues, induces differentiation and mineralization of mature osteoblasts.

osteoblasts. Since Tmem119 is a parathyroid hormone-responsive-Smad3-related factor and interacts with Smad1/5 and Runx2 in osteoblast differentiation (Hisa, 2011), Tmem119 may play a critical role in the commitment of myoprogenitor cells to the osteoblast lineage in muscle ossification (Figure 2). Further analyses of muscle-derived local factors that may regulate muscle ossification are in progress. Moreover, we investigate the role of bone resorbing cells in the pathogenesis of FOP in the present time.

HUMORAL FACTORS LINKING MUSCLE TO BONE

Muscle tissues produce local or systemic growth factors, which have some effects in bone tissues. For example, IGF-I and IGF-binding protein-5 are secreted from muscle tissues. These findings raise the possibility that there might be some humoral factors that are produced in muscle tissues and affect bone in an anabolic fashion. We hypothesized that the signal suppressed by the conversion of muscle tissues into bone might give us a clue to find muscle-derived bone anabolic factors because those factors could be predominantly expressed in muscle tissues, compared with their expressions in bone, and their systemic effects through blood could be more important than their effects in muscle tissues. We therefore selected several factors that exhibited decreased expression levels upon ALK2(R206H) expression using comprehensive DNA microarray analysis (Tanaka, 2012b). Osteoglycin (OGN) is the seventh member of the small leucine-rich proteoglycans, and may be the mechanosensitive gene that mediates an anabolic response of mechanical loading. The levels of osteoglycin as well as the effects of the conditioned medium from OGN-modulated myoblastic cells were found to be positively correlated with osteoblast phenotype and mineralization in osteoblastic cells, although these factors

seemed to reduce osteoblast differentiation in osteoblasts at the early differentiation stage and myoblasts (Figure 3). Moreover, OGN is detected in human serum. These findings suggest that OGN may be crucial humoral bone anabolic factor that are produced from muscle, although clinical studies and in vivo studies using muscle-specific gene-deleted or transgenic mice are necessary. We also found that FAM5C might possess the activity in similar with OGN (Tanaka, 2012c).

ROLE OF FIBRINOLYTYC SYSTEM IN BONE REGENERATION

The clarification of the details in mechanism of bone regeneration is necessary to meet the clinical demand of bone generation for the treatment of bone defects. Plasminogen, a critical component of the tissue fibrinolytic system, activates tissue proteolytic system, including matrix metalloproteinases, and several reports indicate that it mediates tissue repair in skin and liver. We investigated the processes of bone repair in mice with gene deficiency of plasminogen (Plg-/-) and their wild-type littermates (Plg+/+) (Kawao 2013). The bone defect was repaired on day 14 in Plg+/+ mice, but still remained in Plg-/- mice, as assessed by qCT. In Plg+/+ mice, but not Plg-/- mice, blood vessels were observed at the damaged site from day 4, which was associated with the expression of vascular endothelial growth factor (VEGF). The area of cartilage matrix was reduced on day 7 in Plg-/- mice, compared with Plg+/+ mice in alcian and toluidine blue stains. Alkaline phosphatase (ALP) or Osterix-positive cells were localized surrounding the bone tissue at the damaged site on day 7 in Plg+/+ mice. However, the number of ALP- or Osterix-positive cells was decreased at the damaged site on day 7 in Plg-/- mice. These data indicate that plasminogen plays a critical role in the processes of bone repair presumably through the expression of VEGF. Further studies are in progress to investigate the roles of the other fibrinolytic system-modulating factors, such as plasminogen activators and their inhibitors in bone regeneration. The modulation of the tissue fibrinolytic system might be a new strategy for the enhancement of fracture healing and the development of bone regeneration.

CONCLUSION

The links of muscle to bone are not fully understood at the present time. However, this field may be important and interesting physiologically and pathologically. Moreover, the progress of research on the interactions between muscle and bone will give us some clues for the development of novel drugs for osteoporosis and sarcopenia.

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Poster Session

A-1~A-10: Dental Education
B-1~B-32: Frontiers of Biological Science in Dentistry
C-1~C-10: Latest Trends in BioDental Engineering
D-1~D-23: Oral Health and Clinical Treatments

■*Airlangga University, Indonesia*

■*Chulalongkorn University, Thailand*

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

■*University of Hong Kong, China*

■*University of Indonesia, Indonesia*

A-1
05-3

A new integrated Bioethics and Medical Laws course for Health science Students at University of Indonesia (2013)

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BACKGROUND : Since 2012, University of Indonesia started to carry out a new integrated Bioethics and Medical Laws course for the BDS degree in Health science. The course aim was encouraging the students to study about bioethics and medical laws in the consistent manner and learn to respect the responsibility as medical team.

STUDY OBJECTIVE : To find out the most common reasons and problems in dental students who took this new module.

METHODS : Students (316) from 3 branches of health science (dental, nursing, pharmacy) was divided to 16 class The instructional method was Small group discussion, Role play and Card game. Eighty two dental students was selected. Seventy eight students (age 18-20) completed the questionnaire (response rate 83%).

RESULTS : Almost dental students (94.45%) understood the aims and goals of the course clearly. Students think (92%) it is worthwhile studying this integrated course They also (92.40%) rating this course as satisfactory, but (5.44%) still rate poor. As 91.54% felt the course intellectually stimulating, 9,86% disagree with this point. Only 25.3% of the students stated assessment requirements were made clear. There is 43.63% students felt that facilitator can inspire them to learn more and 87.25 % think the facilitators treat the students with respect. During discussion, 61.5% said they have to speak more than 3 times, 47.36% feel unprepared and the rest (52.63%) just have a few problems during the discussions. Majority of the students (98.24%) felt excellent to satisfactory about this strategy, but 14.03% has fear of looking stupid.

CONCLUSION : This course can critically stimulate the student to learn ethics and medical laws, also a lot of information others health profession. Almost dental students felt this course as satisfactory and could widen their knowledge, motivate what to do in the future. At last, the students suggested some facilitators must improve their performance, and the module must be more organized.

Key words : Integrated Bioethics & Medical Laws course, Health Science Students, facilitator

A-2
07-1

The correlation of Indonesian dentist competency-based examination to grade point average and length of study in Dentistry Faculty, University of Jember, Indonesia

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BACKGROUND : In order to protect the public from incompetent health workers, the Indonesia Government through the Health Regulation No. 29 year 2004 requires that each graduate of dental education institutions to take Indonesian Dentist Competence-based Examination (IDCE) as a requirement of obtaining a license to practice. It has been started since 2007. Faculty of Dentistry, University of Jember is one of the educational institutions that have produced dentists since 1995 and its graduates regularly follow IDCE.

OBJECTIVE : To figure out whether the dentists who have been passed IDCE (competent), are correlated with their Grade Point Average (GPA) and length of study at the Faculty of Dentistry University of Jember.

METHODS : The data were achieved from Indonesia Dental Collegiums and Academic Division of Faculty of Dentistry University of Jember year 2012.

RESULTS : The result showed that the graduates of Faculty of Dentistry University of Jember in 2012 were 73 people with the highest GPA was 3.46 and the lowest was 2.54, the average length of study was 6 years 9 month 16 days. The highest score was 69 and the lowest 37 in the scale of 0-100.

CONCLUSION : The result of competency test was not correlated to GPA (R= 0.116) and Length of study (R=0.071), however, almost the incompetent dentists in IDCE were the dentists with GPA lower than 3.00.

Key words : examination, Indonesian, competence-based, Jember University

A-3
09-3

Effect of using "Border mold man" as an instruction media in a step of patient's treatment process, border molding, of the 4th years of dental student (KKU)

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BACKGROUND AND RATIONALE : The conventional instruction media that used in a step of patient's treatment process, border molding in complete denture laboratory are the study casts. The students cannot practice skills like muscle molding in the patients. Therefore this instruction media consists of a man silicone model which called "Border mold man model". It stimulates details of edentulous areas in oral cavity together with video that demonstrates processing of border molding.

STUDY OBJECTIVE : To study the efficiency of using "Border mold man" as an instruction media in a step of patient's treatment process, border molding, of the 4th year dental student.

DESIGN AND EXPERIMENTAL METHODS USED : The volunteers were 28 4th year dental students in 2012, Faculty of Dentistry, Khon Kean University. They were separated into two groups by a random allocation: a control group and an experimental group. Each group was fourteen volunteers. First, both groups attended the lecture about border molding. Second, the control group practiced processing of border molding with a study cast as same as the step in complete denture laboratory and the experimental group practiced with the "Border mold man instruction media". Then, both groups practiced processing of border molding upper arch in complete denture patients. Their skills of border molding were evaluated by a maxillofacial prosthodontist using rubric score 1 (Unacceptable) 2 (Need Improvement) 3 (Acceptable) 4 (Excellent).

RESULTS : The Mann-Whitney-U test demonstrated a statistical in significant difference between the control group and the experimental group (p-value> 0.05). However, comparing a median score of border, retention and stability of the results found that the experimental group had the median score 3.00 **Quartile deviation** 1.25 more than the control group median score 2.00 **Quartile deviation** 1.00.We interviewed the experimental group by recording video. The video revealed that they understood the media very well and they could recall the steps of border molding especially border molding skill.

CONCLUSION : "Border mold man model" is one of the media that help the students to practice skills like muscle molding in the patients.

A-4
12-2

Perceptions and experiences in learning anatomy among Sri Lankan dental students

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Students' perceptions and experiences of learning anatomy should be important in reforming curriculum and designing studying materials. This study was carried out among first year dental students (74) of University of Peradeniya Sri Lanka. Students' learning methods (LM) were studied using a Likert-style questionnaire. *The* "best approach to learning anatomy" and "learning resources" *were* investigated using two open-ended questions. Marks of theory, practical components and final aggregate of anatomy in the first BDS (Bachelor of Dental surgery) examination were compared among students who displayed different types of LM using ANOVA.

Response rate was 80%. Three predominant types of LM were identified; memorising (05) 8.5% (Group I), understanding and memorising (23) 39% (Group II) and visualizing and understanding (31) 52.5% (Group III). Mean marks of every test component in groups II and III were higher than that of the Group I while Group III showed the highest performance. However, these differences were not statistically significant.

Regarding best approach to learning anatomy, all students suggested more than one method. Forty-six students indicated that reading was needed before practical work. Forty-one stated that dissecting and identifying anatomical relations in the cadaver were needed. Twelve students mentioned that studying anatomy in relation to clinical scenarios was interesting. Other methods were reading textbooks and studying good diagrams (27), discussion with others (17), making short-notes (14), observing prosected specimens (13), and attending lectures (10) and tutorials (09). The commonest learning resources were atlases (51), followed by textbooks (44), short-notes (21), computer and internet based material (19).

Our results indicate that our sample of students exercise all 3 types of LM while the predominant type is "visualizing and understanding". Students suggest multiple learning methods as the best approach to learn anatomy. Majority believes that pre- reading and dissections guided by good diagrams are important in successful learning.

A-5
08-1

Examining the relation among training performance, SCE and written examination scores of oral hygiene students in Taiwan

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OBJECTIVES : The purpose of this study was to examine the relation between the Objective Structured Clinical Examination (OSCE) scores, training Performance, and written examination scores of oral hygiene students.

MATERIALS AND METHODS : After a clinical course, 20 fourth-year oral hygiene students were assessed using the OSCE and clinical training performance checklists. The five OSCE stations consisted of one patient communication and four clinical skill scenarios. The compiled written examination scores from the first to third years of courses related to scenarios in OSCEs were also analyzed. A Pearson correlation coefficient was used to compute the relation between these scores.

RESULTS : The OSCE tutors' and the standardized patient's (SP) evaluation scores were not significantly correlated with the written examination scores of the related coursework in the first to third years of school. At the patient communication station, the evaluation scores of the tutor and SP were significantly related to the training performance scores.

CONCLUSIONS : This study found training performance in oral hygiene students could be measured through the use of OSCE, which appears to be a better preparation tool than written examination.

A-6
18-7

Questionnaire survey on student's opinion about dual linguistic education system at Faculty of Dentistry Hiroshima University

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BACKGROUND : International Dental Course Program is an innovation of dental program in Hiroshima University for establishing dental education in 21th century and to develop Asia-based global collaboration in dental education and researches. Until this second year of the program, we received 6 students from 3 sister schools (Indonesia, Vietnam and Cambodia).

For the implementation of the International Dental Course, we developed a Japanese - English dual linguistic education system where all the lectures given in two languages, Japanese and English. We hope this system will not only help the international students to understand the lecture but also increase the English capability of Japanese students. This study is a questionnaire survey with the purpose to analysis the student's opinion about dual linguistic system after they finished their last semester at grade 2.

METHODS : At the end of semester 4, 52 students from second grade (49 Japanese students and 3 international students) filled a questionnaire that was design to provide information about their opinion to dual linguistic system.

RESULTS AND DISCUSSION : The results of this study showed that almost all the students could understand the contents of the lecture that explain by English and Japanese finally (87%). Even if the students could understand the lecture, actually they still have a problem. For international students, it was difficult to understand Japanese parts of lecture explanations; on the other hands it was difficult for Japanese students to understand English explanations. Some students pointed that understanding of the lecture was depend on the lecturer. Almost all of the students think that dual linguistic system is very important and useful for them especially in the relation with internationalization and globalization.

CONCLUSION : Dual linguistic education system is very useful and important both for Japanese and international students at Faculty of Dentistry Hiroshima University even if they still have problems to understand the lecture explanation using this system.

A-7
18-8

Analysis of motivations and assessments from HUD students on Short-term Visit Programs 2012

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BACKGROUND : Hiroshima University, Faculty of Dentistry (HUD) has actively made ceaseless approach to develop Asia-based global collaboration in dental education and researches from undergraduate level. During last decades, we have exchanged many undergraduate students with short exchange programs. Since fiscal 2011, HUD has welcomed international students into our undergraduate school and started a four-year international dental course collaborating with Asian dental schools.

To enhance these approaches, HUD conducted "10days Short-term Visit (SV) Programs 2012" with sister schools supported by Japan Student Service Organization scholarship. With the programs, HUD undergraduate students visited the sister school in fiscal 2012.

SUMMARY OF WORK : Total twelve HUD undergraduate students joined the SV programs. Two students went to Wonkwang University (March 5th-14th, 2013), five students went to Taipei Medical University (March 11th-20th, 2013) and the last five students went to Airranga University (March 11th-20th, 2013).

In this study, we analyzed the motivations and assessments from the SV program students with their essays and assessed the SV programs.

SUMMARY OF RESULTS : As motivations, many of the students pointed "learning dentistry and culture in each places" and "seeing the differences of dental status". Moreover, some of them had "yearnings for going abroad" and "hopes to make friends."

Through the programs, all of the students answered that they could get new insights not only on the dentistry but also on culture, social status, hospitality etc. Furthermore, several students had found their new way to next steps in their career. On the other hand, many of them noticed that their English skill was not enough to discuss about the topics in special fields. Also, it is difficult for several junior students to compare the clinical status rigorously with Japanese one during staying periods.

CONCLUSION : The SV programs made not only the friendship between the institutions closer but also students can understand the importance of mutual understanding, multicultural community and experience harmonious coexistence. The results also suggested that the students need to prepare more about language skills and understand about their own background before going abroad.

We would like to thank all concerned for their understanding and cooperation.

A-8
18-9

Short-term Visit Program 2012 —Visiting Airlangga University—

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BACKGROUND : Short-term Visit (SV) Program 2012 was conducted from 11th to 20th of March 2013 at Faculty of Dentistry, Airlangga University to cultivate students who will lead Asian dentistry.

As a future partner in the dental-medical field, understanding current situations or problems of dental-education and dental-treatment mutually is necessary. Therefore we made a presentation of Japanese dental-medical education and observed how Indonesian students train at the clinic. Besides the academic exchange, we had cultural interaction with Airlangga students.

To evaluate our SV program objectively, we carried out a questionnaire survey at the end of the program to Airlangga students, who supported our SV program and spend most of time with us.

RESULTS : During the SV program, we observed Airlangga University hospital clinic and attended some lectures. Then we found several similarities and differences between Japan and Indonesia on dental-education, dental-treatment, and students' attitudes toward study. Besides the academic exchange, we had cultural interaction with Airlangga University students and fostered global understanding.

As regards the questionnaire we carried out, we got 8 answers. The result showed that impression of Airlangga University students for "Japan" improved significantly and their feedback on our visit was very positive. SV program was consisted of 10 days. Most students rated the length of our visit as adequate.

CONCLUSION : Through SV program, we built an international good friendship and enhanced a mutual understanding with Airlangga University students, which let us became like a big family beyond nationality. We're going to keep our friendship network so that we can exchange information regularly and motivate each other.

With the experience of SV program, some of us became to desire to work in another country as a dentist after graduation. Airlangga University is now one of our options for our future.

A-9
18-10

Short-term Visit Program 2012 —Visiting Taipei Medical University—

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BACKGROUND : Hiroshima University (HU) and Taipei Medical University (TMU) are sister schools. Focused on dental undergraduate level, both of us have actively developed collaboration in dental education through exchange programs. With several kinds of these programs, many students of Faculty of Dentistry, TMU (TMUD) come to Faculty of Dentistry, HU (HUD) every year. To make the students understand Asian dentistry deeply, HUD attempted the 10 days Short-term Visit (SV) Program for undergraduates with TMU in fiscal 2012.

SUMMARY OF WORK : To make a plan for the SV program, we could make contacts and communicate with faculty members of TMUD, under HUD support. Finally, we could decide contents for the program.

This SV program started on March 11th with the opening ceremony in TMU. In this program, we joined a class of Pediatric dentistry with TMU students and visited two dental practitioners in Taipei city. Furthermore, we visited TMU hospital, Wan Fan hospital and Shuang Ho hospital. We could see many departments of dentistry there; Family, Periodontal, Endodontic, Pediatric, Special needs dentistry and even operation wearing scrubs.

During free time and weekend, students of TMU took us many famous and traditional places around Taipei (Jufun, Tamsui, Night Market etc.).

SUMMARY OF RESULTS : We could know some differences between Taiwanese and Japanese hospitals such as insurance system, popular dental treatment, backgrounds of diseases and so on. Furthermore, the status of dental hygienists was different.

During this program, we were always moved to tears with worm hospitality by people in Taiwan including TMU undergraduate students. They are extremely supportive for us.

CONCLUSION : Through this program, we could make the friendship more deeply with undergraduates in Taiwan. Moreover, we could learn the importance of realization for social background. The experiences in Taiwan made us understood ourselves deeply and expanded our world.

A-10
18-11

Short-term Visit Program 2012 —Visiting Wonkwang University—

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BACKGROUND : Faculty of Dentistry, Hiroshima University (HUD) and College of Dentistry, Wonkwang University are partner schools since 2000. HUD had welcomed several students from Wonkwang University. To develop the collaboration among the two schools, HUD attempted the 10 days Short-term Visit (SV) Program for undergraduates with Wonkwang University in fiscal 2012.

SUMMARY OF WORK : We, two undergraduates of HUD joined the SV program with Wonkwang University. After a brief guidance for the program at HUD, we could communicate with faculty members and students of Wonkwang University to make a program contents under HUD support.

The SV program in Republic of Korea started on March 4th. At College of Dentistry, Wonkwang University, we joined a clinical conference of pathology, dental biomaterials exercise. Also, we visited Wonkwang University Daejeon dental hospital and Wonkwang University general hospital in Iksan and observed a jaw surgery, implant operations, dental cares for children and so on. Moreover, we had an opportunity to make a presentation about HUD and to introduce Japanese culture, tea ceremony. As activities with members of Wonkwang University, we visited many traditional places (eg. Jeonju, Buyeo) in Republic of Korea and learn Korean history.

SUMMARY OF RESULT : The SV program was carried out with English. They used several textbooks written in English in Wonkwang University. We noticed that people we met used English very fluently. During the program, students of Wonkwang University help us.

CONCLUSION : This collaboration was effective for us to learn not only their dental status but also relationship between Japan and Republic of Korea on culture and history. Through this program, we could realize the importance of English for language communication. We noticed that we still have many things to learn to be a future dentist in globalization era. We appreciate all the support we received from everyone.

B-1
01-1

Antioxidant effect of Nigella sativa extract in various concentration with DPPH free radical scavenging assay

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BACKGROUND : The gingival epithelium comprise the epithelial tissue that covers the external surface of the gingiva especially junctional epithelium as well as barrier for the bacterial invasion and periodontopathogen products. Gingival epithelium as the first barrier in the periodontology disease progression. One of nature products is ***Nigella sativa***, which common as medicinal plants. Nigella sativa is an aromatic plant belonging to the family Ranunculaceae. Several biological activities have been reported in Nigella sativa seeds, including antioxidant.

PURPOSE : In this context we tried to estimate the antioxidant activity of various concentration prepared from Nigella sativa extract with free DPPH radical scavenging activity.

EXPERIMENTAL METHODS : *Nigella sativa* extract during manufacture from 2500 gram powder of *Nigella sativa* added with 6000 ml ethanol 80%. *Nigella sativa* extracts were made in some consentrations 0,5%, 1%, 2%, 3%, 4%, 5%, 6%,7%, 8%, 9%, dan 10%. Samples were added into buffer solution and 0,5 ml DPPH solution. UV spectrophotometer can measure the intensity of absorption and convert according the formula. The radical scavenging assay was conducted as described by Mansouri et al. The DPPH solution was prepared by dissolving 2.5 mg DPPH in 100 ml of methanol. 25µl of extract or standard antioxidant (quercetin, BHT) were added to 975µL of DPPH solution. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature and the decreases in the absorbance values were measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the following equation.

RESULTS : These findings suggest that *Nigella sativa* extract concentration 0,5% - 2% has shown anti oxidant effect more than 50% and Nigella sativa extract above 3% concentration has shown anti oxidant effect 100%.

CONCLUSION : Nigella sativa extract above 3% concentration has more anti oxidant. Based on this research, nigella sativa extract as addition in the periodontal therapy.

B-2
01-2

The role of TGF-β1 in alveolar bone resorption with Apical Periodontitis

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BACKGROUND : Nowadays Apical Periodontitis and closely associated with inflammatory alveolar bone resorption still an important problem. One of the bacteria causing Apical Periodontitis is *Porphyromonas gingivalis* (*Pg*). Component of the *Porphyromonas gingivalis* such as lipopolysaccharide have an ability to stimulate production of cytokines such as TGF-β1 that promote in both inflammatory bone destruction or remodeling. However, the role of TGF-β1 in alveolaris bone resorption remains unclear.

PURPOSE : The aim of this study was to analyze role of TGF-β1 in alveolaris bone resorbtion based on molecular point of view.

METHOD : Twenty one on tree group male Rat Wistar were conducted. Group (P1) inducted LPS *Pg*, group (P2) get just LPS *Pg* solution and (Po) as control. Apical Periodontitis induced by intrapulpal injection LPS on first upper molar. Periapical tissue samples were taken after three week for each group. Analysis of cytokine expression using immunohistochemical method.

RESULTS : The data was analysis by Anova (SPSS 13.0). The results by statistic analysis were showed expressions TGFβ1 in group P1 were significantly different between group P2 p = 0,001*(p<0,05).

CONCLUSION : Macrophage expressing TGFβ1 may play an important role in reducing the destructive mediators in periapical lesions and in the activation of new bone formation during the healing process of apical periodontitis.

Key words : Apical Periodontitis, LPS, bone resorption-remodeling, TGFβ1

B-3
01-5

The antifungal effect of *Stichopus hermanii* extract to *Candida albicans* in vitro

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BACKGROUND : Sea cucumbers have long been used for food and folk medicine in the communities of Asia. Regarding to the bioactive compound, some species of sea cucumber have been known to have the biomedical properties as antifungal agent. Oral candidiasis is the most common fungal infection in oral cavity caused by *Candida albicans*. An antifungal agent of natural resource will add the great value on the therapy of oral disease. In this preliminary study, golden sea cucumber (*Sticophus hermanii*) was examined its possible antifungal activity towards *Candida albicans* in vitro.

OBJECTIVE : The aim of this study was to examine the antifungal effect of *Stichopus hermanii* extract to the growth of *Candida albicans*.

MATERIAL AND METHOD : The study was an experimental laboratories research with post test only control group design. Three concentration of *Stichopus hermanii* methanolic extract: 20 mg/mL, 40 mg/mL, 80 mg/mL, were tested its antifungal effect against *Candida albicans* by disk diffusion method. The treatment groups were compared to Nystatin oral solution 100.000 IU/ml as positive control and DMSO 1% as negative control. The antifungal effect was examined by measure the diameter of the clear zone around the disk. Data was analyzed by Anova, followed by LSD test.

RESULTS : The result of this study showed the clear zone around the disc of *Stichopus hermanii* extract in all concentrations. It had been proved that antibacterial action of extract *Sthichopus herrmanii* could inhibit the growth *Candida albicans* ($p < 0.05$). The largest diameter of the clear zone around the disc was in the concentration of 80 mg/ mL.

CONCLUSION : *Stichopus hermanii* extract had the antifungal effect against *Candida albicans*. Further in vivo study need to be conducted to explore the potential use of the extract as antifungal agent.

B-4
01-7

The role of hypoxia to apoptosis on bone marrow mesenchymal stem cells (BMSCs) culture for salivary gland defect therapy due to ionized radiation

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BACKGROUND : Salivary gland is one of the normal tissue frequently affected by the side effects of head and neck radiation therapy. One of the side effects is the occurrence of *irreversible* salivary gland defect. The salivary gland defect result in the decrease of saliva production, and in a very severe condition it is called xerostomia. Several studies indicated that the xerostomia causes a reduction in the quality of life of the patients after radiotherapy. A serious attention must be paid upon such a condition, because there is no effective therapy for this condition. The concept of stem cell therapy is one of the new hope as a medical therapy on salivary gland defect. However, the lack of the viability in the form of the survival rate of the transplanted stem cells led to the decrease of the effectiveness of stem cell therapy. The underlying assumption in the decrease of the viability and function of stem cells is an increase of apoptosis incidence. It suggests that the *microenvironment* in the area of the damaged tissues is not conducive to support stem cell viability. One of the *microenvironment* is the hypoxia condition. Several scientific journals stated that the administration of hypoxic cell culture can result in the stress of cells but on the other hand the stress condition of the cells also stimulates the release of heat shock protein 27 (HSP 27) as antiapoptosis through inhibition caspase-9. There is an assumption that HSP 27 is involved in the inhibition of apoptosis in hypoxic conditions in cell culture.

OBJECTIVE : The purpose of this study is to examine the role of preconditioning hypoxia to apoptosis through Heat Shock protein 27 and caspase 9.

METHODS : The experimental design used is exploratory - laboratories. The research procedures are as follows: The isolation is made, culturing the mesenchymal stem cells derived from crista illiaca of the bone marrow from male rabbits. Stem cell culture is performed in hypoxic conditions (O_2 1%-5%) and measured the resistance to apoptosis of bone marrow mesenchymal stem cells by using flowcytometri.

RESULTS : The result of the study that preconditioning hypoxia could decrease amount of apoptotic bodies, increase HSP 27 and decrease level of Caspase 9 significantly ($p < 0,05$).

CONCLUSION : The preconditioning hypoxia could inhibit apoptosis through increasing amount of HSP 27 dan decreasing level of Caspase 9.

Key words : bone marrow mesenchymal stem cells, hypoxia, salivary gland defect

B-5
01-8

Induction HEMA upregulated expression of NLRP3 in rat dental pulp tissue

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BACKGROUND : Adhesive resin is one of the most important groups of materials in dental practice. Most of adhesive resin consist of various methacrylate monomers such as HEMA (2_hydroxyethyl dimethacrylate) and TEDGMA (triethylene glycol dimethacrylate). These methacrylate monomers are responsible for clinical disadvantages and biological adverse. Although some studies showed those components are toxic, but resin based dental material is still used extensively in dentistry. Oxidative stress caused by undesired generation of excess reactive oxygen species (ROS). ROS from unpolymerized monomer like HEMA is the key factor leading to pulp damage. Generation of excess ROS from resin monomer can induce a mechanism underlying cellular reaction as a disturbed response of innate immune system. Nod like receptor (NLR) family members is pyrin domain containing 3 (NLRP3) is the most versatile innate immune receptor. NLRP3 has broad specificity for mediating an immune response to danger signal. We hypothesize that NLRP3 plays essential role in detection of non microbial pathogens and the initiation of inflammation within the dental pulp.

OBJECTIVE : The aim of this study is to evaluate the expression of NLRP3 in normal rat dental pulp tissue that induced by adhesive resin.

MATERIALS AND METHODS : The study was an experimental laboratories research with post test only control group design. 10 rat teeth were treated with adhesive resin then pulp tissue were collected after various time dependent manner, 24, 48 and 72 hours. The expression of ROS and NLRP were examined by enzyme activity test and Elisa.

RESULTS : The result of this study showed that there is a decrease of catalase activity enzyme and displayed high level of NLRP3 protein.

CONCLUSION : NLRP3 plays an important role in dental immune defense against non microbial pathogens such as ROS.

B-6
02-1

Interleukin 12 increased RANKL/OPG expression ratio in human PDL cells

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BACKGROUND : Interleukin 12 (IL-12) is a multifunctional pro-inflammatory cytokines that involved in Th1 differentiation and also play a role in osteoclastogenesis.

OBJECTIVE : The aim of this study was to investigate the osteoimmunology effect of IL-12 on human periodontal ligament cells (hPDLs).

METHODS : Human PDLs were cultured with 0-20ng/ml of IL-12, range for 24-120 hours. The effect of IL-12 on RANKL and OPG mRNA expression was performed by quantitative PCR. The signaling pathway that involved was examined by means of chemical inhibitors.

RESULTS : IL-12 increased RANKL/OPG ratio of mRNA expression in a dose dependent manner. This effect could be observed form 8 hours after IL-12 treatment. Addition of STAT4 and NF-kB inhibitors, but not indomethacin, suppressed the inductive effect of IL-12 on RANKL expression, suggesting the involvement of STAT4/NF-kB signaling pathway. However, these inhibitors didn't show any significant effect on OPG expression. From immunofluorescence analysis, IL-12 could induce NF-kB nuclear translocation. Moreover, application of STAT4 inhibitor could not inhibit the nuclear translocation, suggesting that STAT4 might function downstream of NF-kB.

CONCLUSION : IL-12 regulated RANKL/OPG ratio via STAT4/NF-kB signaling pathway. The result indicated the role of IL-12 in periodontal tissue homeostasis.

B-7
02-2

Genome-wide analyses in yeast model to investigate the mechanisms of *Aggregatibacter actinomycetemcomitans* cytolethal distending toxin.

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BACKGROUND : *Aggregatibacter actinomycetemcomitans*, a periodontal pathogen, secretes a cytolethal distending toxin (AaCDT) that causes host cell cycle arrest and cell death. Although CDT could be an important virulence factor, its mechanism of cytotoxicity is not fully understood.

OBJECTIVE : To investigate the mechanisms of AaCDT by genome-wide screening for host mutations that affect cellular sensitivity to the catalytic subunit, AaCdtB, in a *Saccharomyces cerevisiae* model.

METHODS : We transformed the yeast haploid deletion library, a collection of yeast strains with single gene deletions of virtually all non-essential ORFs in the genome, with plasmids carrying galactose-inducible AaCdtB. Yeast mutants that showed either hypersensitivity or resistance to AaCdtB were selected and rescreened by spotting assay. AaCdtB expression was confirmed by western blot analysis; any strains that showed no or weak expression of AaCdtB were omitted from the analysis. The lists of genes whose mutations confer hypersensitivity or resistance to AaCdtB were analyzed for Gene Ontology (GO) term enrichments.

RESULTS : From approximately 5,000 deletion strains, we isolated over 100 strains that are hypersensitive and over 200 strains that are resistant to AaCdtB. Initial GO analyses indicated that genes involved in DNA damage responses and DNA repair, especially in DNA break repairs, are significantly enriched in the AaCdtB hypersensitive list. This suggests that the major mechanism of AaCdtB in the cells is the generation of DNA breaks. On the other hand, no enrichment was observed in the AaCdtB resistant list, but a wide variety of biological processes including transcription, metabolic processes, chromatin organization, and RNA processing may be involved.

CONCLUSIONS : The screens in the yeast deletion library allowed us to identify host genes required for cell survival upon AaCdtB exposure (hypersensitive mutants) and for AaCdtB cytotoxicity (resistant mutants). Further analysis could lead to more insights into the mechanisms of CdtB intoxication.

B-8
02-3

Antimicrobial activities of Crofton weeds oil (*Eupatorium adenophorum* Spreng) against oral bacteria and fungi

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BACKGROUND : In the era of antimicrobial resistance, herbal medicines are becoming potential alternative treatments. *Eupatorium adenophorum* Spreng (crofton weed) is a perennial herbaceous weed found in several regions of the world. The plant has been used in folk remedies as an antimicrobial, blood coagulant, antiseptic, analgesic and antipyretic. Previous studies revealed its antimicrobial ability against several pathogens. However, there is no evidence of its effect on oral microorganisms.

OBJECTIVE : This study aimed to demonstrate the antimicrobial activity of Crofton weed oil against oral bacteria and fungi.

METHODS : Caries-related bacteria: *Streptococcus mutans* ATCC25175, *Actinomyces viscosus* ATCC15987 and *Lactobacillus casei* IFO3533 and oral *Candida* species: *Candida albicans* ATCC90028, *Candida glabrata* TIMM1098, *Candida tropicalis* ATCC750, *Candida krusei* ATCC6258, *Candida dubliniensis* 16F and *Candida parapsilosis* ATCC20019 were used in this study. The antimicrobial activity against these strains was determined by a disc diffusion method. Additionally, scanning electron microscopy (SEM) was used to investigate microbial ultra-structure alteration after the oil treatment.

RESULTS : The oil exhibited similar antibacterial activity as 0.2% chlorhexidine (0.2%CHX) against all tested bacterial strains. The zones of inhibition produced by the oil against *S. mutans*, *A. viscosus* and *L. casei* were 11.1 ± 1.05 mm., 18.7 ± 1.81 mm. and 10.4 ± 1.55, respectively. However, 0.2%CHX had greater antifungal activity against *C. glabrata*, *C. krusei*, *C. dubliniensis* and *C. parapsilosis* than the oil. Neither reagents demonstrated antifungal effect on *C. albicans*. Unexpectedly, the antifungal effect on *C. tropicalis* of the oil (19.3 ± 1.05 mm.) was nearly two folds higher than that of 0.2%CHX (11.0 ± 0.0 mm.). SEM of microbial cells treated with the oil showed perforations on the cell surface.

CONCLUSION : Crofton weed oil exhibited antimicrobial activities against caries-related bacteria and *Candida* species, except *C. albicans*, by affecting microbial cell surface.

B-9
04-1

New antifungal for oral and systemic candidiasis: in vitro, in vivo efficacy, proteomics and genomics

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BACKGROUND : *Candida* infections are a major problem in compromised host populations like aging patients, transplant recipients, etc. Oral and denture candidiasis affect millions of patients worldwide. Limited number of antifungals and emergence of drug resistant strains have posed a huge clinical challenge. Therefore, aim of our study was to discover and characterize a new antifungal agent from a library of 50,000 small molecules.

METHODS : Initially, small molecule library was screened for yeast-to-hyphal transition inhibitors which led to the discovery of new molecule "SM21". Antifungal activity of SM21 was evaluated comprehensively for clinical strains derived from denture stomatitis and nasopharyngeal carcinoma patients, including that of multi-drug resistant. In vivo efficacy of SM21 was examined by oral and systemic candidiasis mouse models. Safety of SM21 was evaluated using primary cells cultures and in vivo studies. Structure-activity relationship was performed using analogues. Mechanism of the new molecule was investigated using proteomics and microarray approaches.

RESULTS : The new antifungal agent demonstrated better antifungal and anti-biofilm activity than existing antifungal agents and was active against wide-range of resistant isolates. SM21-treated mice displayed significantly less tongue lesions than the control and nystatin-treated mice in oral candidiasis model. SM21-treated mice demonstrated 100% survival in systemic candidiasis model compared to control for which none survived. No detrimental effect of SM21 was observed in vitro or in vivo. Hence, new antifungal agent was effective for treating oral and systemic candidiasis with no adverse effects. Proteomics and genomics studies demonstrated that SM21 targets the fungal cell wall, possibly by HOG pathway molecule PBS2, that subsequently reduces the beta-1,6-glucan in fungal cell wall.

CONCLUSION : We have discovered a new antifungal agent SM21 for oral and systemic *Candida* infections (US provisional patent No: 61733094). This discovery will bring enormous benefits for the patients suffering from ubiquitous *Candida* infections.

B-10
09-2

Evaluation of three medicinal plants for anti-*Streptococcus mutans* activity

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BACKGROUND : *Clausena excavata*, *Clausena harmandiana* and *Clausena lansium* (Rutaceae) are widely distributed in south Asia. They have been used as folk medicines for the treatment of infectious diseases. Dental caries is one of the most prevalent chronic diseases that caused by multiple factors. The evidences have proved that *Streptococcus mutans* play a crucial role in the initiation of dental caries in humans.

OBJECTIVES : This study investigated the effects of *C. excavata*, *C. harmandiana* and *C. lansium* against *Streptococcus mutans*.

EXPERIMENTAL METHODS : The root and leaves of *C. excavata*, *C. harmandiana* and *C. lansium* were extracted by methanol. The crude extractions were dissolved by Dimethyl sulfoxide and diluted by distilled water then test for their antimicrobial activities by disc diffusion method. For broth dilution method, the minimum inhibitory concentration (MIC) was determined as the lowest concentration of methanol extract inhibiting bacterial growth after 24 h of incubation at 37°C. The minimal bactericidal activity (MBC) was evaluated using the viable cell count method. The concentrations below MIC were selected to test the influence on acid production.

RESULTS : Only the root of *C. harmandiana* created an inhibition zone size 8.5 ± 1.58 mm in diameters in comparing to standard antibiotic, 0.12% Chlorhexidine which gave 14 mm. For dilution method, the MIC against *S. mutans* of crude extraction from the root of *C. harmandiana* was 0.125 mg/ml but not shown in killing effect at concentration of 1 mg/ml. The crude extraction from leaf of *C. lansium* had inhibitory and bactericidal effect at a concentration of 0.25 mg/ml and 1 mg/ml, respectively. Additionally, the *C. lansium* had inhibition effect ($p<0.05$) on acid production of *S. mutans* compared with the control group.

CONCLUSION : The crude extractions from root of *C. harmandiana* and leaf of *C. lansium* could be used as potential dental-caries-preventative medicine.

B-11
11-2

Characterization of the different colonies in oral squamous cell carcinoma (OSCC) cell lines

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INTRODUCTION : It is widely accepted that tumours are heterogeneous and this heterogeneity is preserved within cell lines. The aim of this study is to characterize the growth characteristics of different clones in OSCC cell lines to identify possible cancer stem-like cells which might be responsible for treatment resistance and recurrence.

MATERIALS AND METHODS : Two OSCC cell lines (ORL-115, ORL-48) cultured in DMEM/F12 with 10% FBS were plated in different densities (100, 200 or 300 cells) in 6 well plates for 10-12 days before different types of clones (holoclone, meroclone, paraclone) were counted. Colonies from each clone type was isolated and expanded for growth and sphere formation assay. To determine the population doubling time (PDT) of each clone type, 1000 cells were seeded in 24 well plate and counted each day for 10 days. For sphere forming assay, 1000 cells/ml were seeded and spheres were counted after 7 days. Expression of E-cadherin was examined on the different clone types by immunocytochemistry.

RESULTS : Holoclone made up the largest population (67.7%) in ORL-115. In ORL-48, proliferation of holoclone is highest compared to meroclones and paraclones (PDT = 1.14 vs 1.24 and 3.12 days respectively; $p<0.05$). There was no significant difference in the proliferation rate between holoclones and meroclones in ORL-115 (1.32 vs 1.44 days respectively; $p=0.19$) Paraclones from ORL-115 did not survive after isolation. There was no statistical significant in sphere formation between holoclone and meroclone (sphere number = 27 vs 23 respectively; $p=0.06$) from ORL-115. E-cadherin staining was positive for cells within holoclones and meroclones for both cell lines.

CONCLUSION : Distinct colonies with different growth characteristics can be isolated from OSCC cell lines. Both holoclones' and meroclones' ability to continuously proliferate and form spheres affords an opportunity to study the biology of the cancer stem-like cells in OSCC.

B-12
14-1

Application of green fluorecent protein reporter system in *Streptococcus mutans* for study on dual-species biofilms

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BACKGROUD : *Streptococcus mutans* (*S. m*) has been well characterised as a primary cariogenic pathogen in dental plaque which known as typical mixed-species biofilms. Although the previous studies investigating *S. m* in single-species biofilm development have certainly advanced our knowledge of the microbial behaviours, a deeper and clearer understanding regarding the mechanisms of multi -species biofilm is needed. Previously, we had established a constitutive green fluorescent protein (GFP) reporter system in *S. m* and proved its use as a metabolic activity indicator in the single- biofilms of *S. m*.

OBJECTIVES : To construct GFP synthesis of two different strains of *S. m* and study the antibiotic resistance of *S. m* growing with *Streptococcus. gordonii* (*S. g*) in dual-species biofilms.

METHODS : Single- and dual-species biofilms were formed by *S. m* UA159, C67-1 and *S. g* ATCC35105 respectively. Biofilms were treated with 0.001~0.01% chlorhexidine for 4 h. Biofilm formation and treatment efficacy were evaluated by fluorescence intensity (FI) and resazurin metabolism assay. Morphology of the biofilms were observed under fluorescent microscope.

RESULTS : A linear correlation was obtained between FI-increase and metabolic activity in *S. m* single-species biofilms. In the range of treatment concentration 0.004~0.008%, significant dose-response relationships were shown between the inhibition of GFP expression and concentration both in single- and dual-species biofilms. When co-cultured with *S. g*, the biofilm formation of both *S. m* strains decreased. However, the antimicrobial resistance of *S. m* displayed distinct strain dependence. Decreased resistance was observed in UA159, while increased resistance was found in C67-1. Compared with single-species biofilms, the morphology of C67-1 in dual-species biofilms showed more aggregating. For UA159, there is no significant morphology changes observed in single- or dual-species biofilms.

CONCLUSIONS : GFP synthesis can be used as a species specific marker in dual-species biofilm and can reflect the antimicrobial resistance of the targeted species.

B-13
15-1

In vivo bone regeneration of bone morphogenetic protein-2 and Ling-Zhi protein on novelty biodegradable polymer scaffold

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BACKGROUND : Bone morphogenetic protein-2 (BMP-2) is an osteoinductive protein. Although clinical evidences confirmed the regulation of human immunity and promotion of diabetic wound healing, no study related to effect of Ling-Zhi protein on bone was investigated. Also, carriers were needed as delivery systems/scaffolds for these proteins.

OBJECTIVES : This study is (1) to evaluate the biocompatibility of the Ling-Zhi protein and biodegradable polymer scaffold (BPS), and (2) to determine the osteogenetic ability of the Ling-Zhi protein compared with BMP-2 in bony defects of the rabbits.

EXPERIMENTAL METHODS : Twelve male New Zealand white rabbits (18-24 weeks, 3.3-3.8 Kg) were used in this study. Three standardized bony defects were created in the nasal bone for each rabbit. Saline, BMP-2 and Ling-Zhi protein were carried by BPS and were inserted into the defects, respectively. Animals were followed for 1, 2, 4 and 8 weeks. Three rabbits were sacrificed at each observation ending and specimens were obtained for radiographic and histomorphometric evaluation.

RESULTS : Radiography revealed new bone formation in all groups at each follow up period. The width and volume of new bone deposit increased significantly in BMP-2 group at 4-week follow-up ($p<0.05$). The saline group had the least bone regeneration in width compared to other groups in week 8 ($p<0.05$). Histomorphology revealed no hyper-inflammation in all samples. The regeneration/maturation of bone in the BMP-2 group was superior to other groups at each observation point. The maturation of newly formed bone in Ling-Zhi protein group shows better result than the saline group.

CONCLUSION : BPS as a carrier of BMP-2 or Ling-Zhi protein was biocompatible. BMP-2 and Ling-Zhi protein exhibited potential of osteogenesis and facilitate maturation of newly formed bone in the rabbits.

B-14

18-1

The functional role of MSX1 in stem cells from human exfoliated deciduous teeth (SHED)

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BACKGROUND : Stem cells from human exfoliated deciduous teeth (SHED) are a promising source in regenerative medicine. Until now, many papers have reported SHED show osteogenic/odontogenic differentiation. However, the mechanism is not fully understood.

Previously, we found that the expression level of MSX1 is significantly higher in SHED than in fibroblasts and bone marrow MSC. Msx1 is homeobox transcription factor and very important for body development including tooth morphogenesis.

Thus, the hypothesis is that MSX1 is involved in osteogenic/odontogenic differentiation as transcription factor mediated some target gene in SHED.

AIM AND DESIGN : To study the roles of MSX1 in SHED, we identify the MSX1 target genes using geneme-wide ChIP-sequencing (ChIP-seq) and DNA microarray, and examined the effects of MSX1 knockdown on mineralization.

RESULTS : ChIP-seq data showed 7249 MSX1-binding sites ($P<10^{-5}$). In DNA microarray analysis, 1009 genes were significantly down-regulated or up-regulated by MSX1 knockdown ($P<0.05$, fold change>1.5). For the target genes, we combined the data of ChIP-sequencing and DNA microarray. Consequently, 109 overlapping genes were obtained. Among 109 genes, many genes involved in osteogenic/odontogenic differentiation were found. Moreover, osteogenic/odontogenic differentiation potency was lost in MSX1 knockdown cells.

CONCLUSION : MSX1 acts as a positive regulator of osteogenic/odontogenic differentiation in SHED, likely via regulating expression of its target genes.

B-15
18-2

Regulation of the Na^+-H^+ exchanger activity associated with bicarbonate secretion from rat salivary ducts

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BACKGROUND : Salivary ducts secrete bicarbonate. Parasympathomimetic agents induce an increase in the intracellular Ca^{2+} concentration, and subsequent bicarbonate secretion through the Ca^{2+}-dependent Cl^- channel. Simultaneously H^+ ions are extruded by the Na^+-H^+ exchanger (NHE). Sympathomimetic β agonists also evoke bicarbonate secretion via an increase in the intracellular cAMP concentration and subsequent activation of the CFTR Cl^- channel. However, the regulation of the NHE activity in salivary ducts remains obscure.

OBJECTIVE : To characterize the activity of the NHE during bicarbonate secretion from rat salivary ducts, we measured intracellular pH changes induced by Ca^{2+} and cAMP signals.

EXPERIMENTAL METHODS : Parotid glands isolated from male Wistar rats were minced and incubated with collagenase to separate acini and ducts. The intralobular duct segments were loaded with the pH-sensitive fluorescence dye, BCECF. The intracellular pH changes were measured with the ARGUS-HiSCA system.

RESULTS : Application of the Ca^{2+}-increasing agent, carbachol (CCh), did not affect the intracellular pH, while forskolin+IBMX, cAMP-increasing agents, significantly decreased the pH. The NHE inhibitor, 5-(N,N-dimethyl)amiloride (DMA), reduced the pH, suggesting that the NHE is at least partially activated in the resting state. In the presence of DMA, addition of CCh and forskolin+IBMX markedly decreased the intracellular pH compared with the effects in the absence of DMA, indicating that the NHE was activated during bicarbonate secretion induced by the stimulations. The carbonic anhydrase inhibitor, methazolamide, did not significantly change the pH. When bicarbonate and H^+ were at an equilibrium state under the inhibition of the carbonic anhydrase by methazolamide, application of CCh significantly increased the pH, while forskolin+IBMX did not. Moreover, CCh restored the pH level that had been reduced by forskolin+IBMX in the absence of methazolamide.

CONCLUSION : Results obtained suggest that the NHE is strongly activated by CCh due to the high pH set point, which is not affected by forskolin+IBMX.

B-16
18-5

P2X7 receptor and cytokines contribute to extra-territorial facial pain following a trigeminal nerve injury

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BACK GROUND : It has been reported that the whisker pad (WP) area, which is innervated by the second branch of the trigeminal nerve, shows tactile allodynia/hyperalgesia following transection of the mental nerve (MN: the third branch of the trigeminal nerve). However, the mechanisms of this extra-territorial pain induction still remain unclear. Recently, we reported that P2X7 receptor (P2X7R), an ionotropic ATP receptor, in microglia facilitates perception of noxious input with cytokines.

OBJECTIVES : This study was designed to examine a possibility if P2X7R, in cooperation with cytokines, contributes to the induction and spread of allodynia/hyperalgesia at non-injured skin territory.

EXPERIMENTAL METHODS : Rats were anesthetized, and MN was transected. A438079 (i.t., 35 μg/rat), a P2X7R antagonist or etanercept (i.t., 5 or 50 ng/rat), a tumor necrosis factor (TNF)-α receptor-binding recombinant drug, was infused intrathecally. The tactile allodynia/hyperalgesia was assessed by von Frey filaments.

RESULTS : One day after MN transection, tactile allodynia/hyperalgesia was developed on the ipsilateral WP area, which is in the non-injured skin territory. The tactile allodynia/hyperalgesia lasted for more than 6 weeks. In response to MN transection, activation of microglia sustained for 6 weeks after MN transection. Up-regulation of P2X7R, membrane-bound TNF-α (mTNF-α), soluble TNF-α (sTNF-α) and phosphorylated (p)-p38 mitogen-activated protein kinase (MAPK) in the trigeminal sensory nuclear complex (TNC) were induced up to 6 weeks after MN transection. Allodynia/hyperalgesia at 24 hours, 3 days, 1 week, 3 weeks and 6 weeks after MN transection was suppressed by etanercept. A438079 also attenuated tactile allodynia/hyperalgesia or up-regulation of mTNF-α, sTNF-α and p-p38 MAPK in the TNC.

CONCLUSIONS : Based on these findings, sTNF-α released by microglia via P2X7R activation plays an important role in not only the initiation, but also maintenance of extra-territorial allodynia/hyperalgesia after MN transection.

B-17
18-12

Soluble Klotho does not rescue but rather exaggerates skeletal defects in Klotho-deficient mice.

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Klotho-deficient mice suffer from a syndrome resembling accelerated human aging, including skeletal and dental impairments and chronic renal failure. Klotho is a type I transmembrane protein and expressed in restricted tissues such as kidney, parathyroid and choroid plexus, while its truncated form in circulation (soluble Klotho, sKL) may be involved in several biological functions. Membrane Klotho forms a complex with fibroblast growth factor (FGF)23 and FGF receptor (FGFR). FGF23 is expressed primarily in osteoblasts/osteocytes and acts on kidney as a phosphaturic hormone. An excess amount of circulating FGF23 decreases serum phosphate levels and thereby causes hypomineralization in bone. We then assessed whether the recruitment of sKL rescues renal and skeletal defects in Klotho-deficient mice. Chronic administration of sKL to young male Klotho-deficient mice did not alleviate the symptoms of hyperphosphatemia, hypercalcemia, hypervitaminosis D and FGF23 overproduction. Also, there was no improvement in the expression of the FGF23 target genes *Slc34a1/3* and *Cyp27b1* in kidney. However, sKL exaggerated Klotho-deficient skeletal phenotypes, such as a reduction in growth plate width and mineral apposition rate. sKL-FGFR-FGF23 complex formation was observed in bone but not in kidney of Klotho-deficient mice, when treated with sKL. sKL decreased mRNA levels of *Phex* (phosphate-regulating gene with homologies to endopeptidases on the X-chromosome) and *Mepe* (matrix extacellular phosphoglycoprotein), both of which appear to be involved in skeletal defects. The sKL-dependent down-regulation of *Phex* and *Mepe* in bone of Klotho-deficient mice was abrogated by anti-FGF23 antibody *ex vivo*. Thus, recruitment of sKL may directly exaggerate skeletal defects via the actions of phosphate regulating factors expressed in bone in Klotho-deficient mice. These results suggest that sKL may impair bone formation at least in patients with renal dysfunction and/or FGF23 overproduction.

B-18
18-13

Osteoclast-like Cells are Involved in Aortic Aneurysm Formation

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Recent advances in the understanding of cardiovascular events in chronic kidney disease, atherosclerosis and aortic aneurysm have revealed that vascular calcification is an active process involving not only vascular cell apoptosis but also bone remodeling. The implication of osteoclast-like cells appears to be involved at least in the later process, while its underlying mechanism remains largely unknown. We therefore investigated the formation of tartrate-resistant acid phosphatase (TRAP) - positive osteoclast-like cells in aneurysm.

First, we used alizarin red S staining to confirm calcification in abdominal aorta from patients undergoing surgical repair for aneurysm and stenosis. We identified osteoclast-like cells in aneurysm but not stenosis. To elucidate the molecular basis of what was observed in aneurysm, we determined gene expression levels of several osteoclast regulatory factors such as RANKL, osteoprotegerin and TNFα. Both immunohistochemistry and Western blotting showed that stronger expression of TNFα in aneurysm than that in stenosis. To understand the induction of osteoclastogenesis in aneurysm, we cultured the mouse macrophage cell line RAW 264.7 in the presence of $CaPO_4$ crystal with and without TNFα, which mimics pathologies of aneurysm and stenosis, respectively. As expected, the formation of osteoclast-like cells and levels of medium MMP-9, a well-known accelerator of aneurysm formation, were significantly increased when treated with $CaPO_4$ plus TNFα. In a mouse model of $CaPO_4$-induced aneurysm, we found that TRAP-positive cells were formed in aneurysm and that a clinical dose of zoledronic acid, a class of bisphosphonates used to treat osteoporosis, remarkably inhibited the production of TRAP-positive cells and the vessel dilatation in the carotid artery.

In conclusion, we uncovered that both stimulations of TNFα and $CaPO_4$ may induce osteoclast-like cells in aortic aneurysm, and that osteoclast-like cells might be an essential therapeutic target for aortic aneurysm.

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18-15

Inhibition of PRIP expression results in impaired adipogenesis

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BACK GROUND : Phospholipase C-related catalytically inactive protein (PRIP) is similar to phospholipase C (PLC)-δ1 but lacks PLC activity. We produced PRIP knockout (PRIP-KO) mice and identified the mice appeared to be a lean phenotype with reduced fat mass, suggesting the involvement of PRIP in lipid metabolism.

OBJECTIVES : To investigate the role of PRIP in lipid metabolism, we examined the effect of high fat diet in obesity.

EXPERIMENTAL METHODS : Six-week-old WT and PRIP-KO mice were fed a high-fat diet for 10 weeks. The concentration of plasma insulin or leptin was measured by each ELISA kit. For mouse embryonic fibroblast (MEF) differentiation, cells were treated with the adipogenic cocktail containing insulin, dexamethasone, IBMX, and rosiglitazone. Two days after the induction, the cells were cultured in maintenance medium (DMEM with FBS) containing insulin during the remaining duration of differentiation. The maintenance medium was changed every 48 hrs. Mouse 3T3-L1 preadipocytes, were also differentiated by the same protocol, but rosiglitazone was not included in the adipogenic cocktail. Intracellular lipid droplets were stained with oil red O. PRIP was knockdown by siRNA methods in 3T3-L1 cells, and adipocyte marker gene expression were measured by quantitative real time polymerase chain reaction (qPCR) and western blot.

RESULTS : PRIP expression gradually increased during adipocyte differentiation in 3T3-L1 cells. PRIP gene knockdown by siRNA in 3T3-L1 cells significantly decreased PPARγ2, Cebp/α, and aP2 expressions measured by qPCR, suggesting impaired adipogenesis. Then, we performed adipogenesis assay using MEF prepared from PRIP knockout (PRIP-KO) mice. Differentiation of PRIP-KO MEF into adipocyte was remarkably inhibited, which was determined by oil red O staining. Furthermore, expressions of adipogenesis markers were suppressed in PRIP-KO MEFs. These results indicated that PRIP regulates adipogenesis process.

CONCLUSION : We show that genetic deficiency of PRIP results in protected against diet-induced obesity in mice, and elevated expression of PRIP associates with adipogenesis. These findings improve our understanding of the molecular mechanisms regulating white adipose tissue formation and ultimately contribute to the development of new tools to treat obesity.

B-20
18-16

Phospholipase C-related catalytically inactive protein modulates insulin secretory vesicle movement

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BACKGROUND : Glucose-stimulated insulin release from pancreatic β-cells shows a biphasic secretion pattern. The first phase insulin release consists of the fusion process of insulin granules that are predocked on the plasma membrane and/or recruited from a readily releasable pool. The second phase release correlates with the mobilization of insulin-containing granules from the releasable pool to the cell periphery, which is mediated by microtubules and a motor protein kinesin-1 (KIF5). We previously reported phospholipase C-related catalytically inactive protein (PRIP) knockout mice exhibit hyperinsulinemia. PRIP interacts with $GABA_A$ receptor-associated protein (GABARAP), a modulator for intracellular trafficking, and regulates the recruitment of $GABA_A$ receptors to the cell surface.

OBJECTIVES : The present study investigated whether PRIP participates in insulin secretion, and a possible mechanism of PRIP-mediated insulin secretion was examined.

EXPERIMENTAL METHODS : Isolated pancreatic islets from *PRIP*-knockout mice and *PRIP*-knockdown mouse insulinoma (MIN6) cells were used for insulin secretion assay. Insulin vesicle movement was observed by time lapse imaging and analyzed by 2D-chemotaxis assay. The association among insulin vesicle, GABARAP and KIF5 was examined using the density gradient centrifugation and immunocytochemistry.

RESULTS : In *PRIP*-knockdown MIN6 cells, the KIF5 motor protein co-localized well with the vesicle marker protein Rab27a- and the phogrin-rich fraction in a density step-gradient analysis. *PRIP*-knockdown also facilitated the mobility of GFP-phogrin-labeled secretory vesicles. Functional reduction of GABARAP, a microtubule-associated protein, with siRNA and the microtubule-dissociation mutant plasmid in MIN6 cells decreased second-phase insulin release and vesicle mobility, respectively. Furthermore, dissociation of PRIP from GABARAP using an interference peptide (GABARAP40-67) enhanced the localization of GABARAP and KIF5 to insulin vesicles and promoted vesicle mobility.

CONCLUSION : Present study demonstrates the complex between PRIP and GABARAP regulates the KIF5-mediated second phase of insulin secretion and provides new insights into insulin exocytosis mechanisms.

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18-17

PRIP1- and *PRIP2*-double knockout mice exhibit resistance to neuropathic pain in a partial sciatic nerve ligation model

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BACK GROUND AND OBJECTIVE : We identified a novel inositol 1, 4, 5-trisphosphate binding protein and termed it phospholipase C-related catalytically inactive protein (PRIP) based on the structural basis for the lack of enzyme activity. PRIP has two mammal isoforms PRIP1 and PRIP2. Both have a number of binding partners including $GABA_A$ receptor associated protein (GABARAP), a modulator for inhibitory neurotransmission. *PRIP1* single knockout mice exhibited a hyperalgesia phenotype compared with wild-type mice due to altered expression of $GABA_A$ receptor subunits. In this study, we further investigated an involvement of PRIP in pain signaling by using *PRIP1*- and *PRIP2*-double knockout (*PRIP*-DKO) mice.

METHODS : Wild-type and *PRIP*-DKO male mice, 10 to 14 weeks old, were subjected to partial sciatic nerve ligation (PSNL) surgery. ddY strain mice, which were injected specific siRNAs of PRIP1 and PRIP2 or the scrambled siRNAs in the spinal cord, were subjected to PSNL surgery. Mechanical allodynia was measured by the von Frey hair test.

RESULTS : The *PRIP*-DKO mice that underwent PSNL surgery indicated decreased pain sensation in the paw withdrawal threshold, indicating the inverse phenotype of pain sensation between *PRIP*-DKO and *PRIP1* KO mice. Expression patterns of $GABA_A$ receptor subunits in the *PRIP*-DKO spinal cord were similar to those of wild-type mice; however, the expression of K^+-Cl^--cotransporter-2 (KCC2) was increased in naive *PRIP*-DKO mice, and it was highly phosphorylated. KCC2 expression in PSNL-operated *PRIP*-DKO mice was remained high level similar to the level of sham-operated wild-type mice. Neuropathic pain induced by PSNL was ameliorated in siRNA-injected *PRIP*-double knockdown (DKD) mice, which was inhibited by intrathecal administration with R-(+)-DIOA, a KCC2 inhibitor.

CONCLUSION : These data indicated that the suppressed expression of both of PRIP1 and PRIP2 induces the elevated expression of KCC2 and results in amelioration of neuropathic pain in *PRIP*-DKO mice.

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18-18

PRIP modulates autophagosomal maturation containing invasive bacteria.

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BACKGROUND : Autophagosome is a degradative pathway characterized by double-membrane containing LC3 (microtubule-associated protein 1 **l**ight **c**hain **3**) triggered by various stimulation including nutrient starvation and bacterial invasion. The membrane vesicles called autophagosomes are responsible for delivering cytoplasmic material to lysosomes. We previously identified GABARAP, a homologous molecule to LC3, as a binding partner of PRIP (**p**hospholipase C-**r**elated catalytically **i**nactive **p**rotein), which is originally identified as an Ins(1,4,5)P_3 binding protein and later on characterized as a modulator for the trafficking of $GABA_A$ receptor. Since PRIP has a possibility of binding to LC3, PRIP could be correlated with autophagic pathway. We elucidated that autophagosome formation in *PRIP*-KO cells is up-regulated (Umebayashi et al., 2013), however, detailed mechanism is not clarified.

OBJECTIVES : From the viewpoint of bacterial invasion-induced autophagy, we investigated if PRIP mediates autophagic system by using *Staphylococcus aureus*.

RESULTS : We visualized autophagosomes by using GFP-LC3 expressing mouse embryo fibroblasts (MEFs). Autophagosomes containing *S. aureus* in the *PRIP*-KO MEFs were larger in size and more predominant in the number per a cell. We then quantified the intracellular proliferated *S. aureus* to see the facilitation of autophagy in the *PRIP*-KO cells, because autophagosome formation increases intracellular *S. aureus* replication. The proliferation of *S. aureus* was up-regulated in *PRIP*-KO cells. Moreover, we clarified that conversion of autophagosomes into autolysosomes were inhibited, and that the bacterial proliferative inhibition was down-regulated in the *PRIP*-KO cells by time-lapse imaging experiments. In addition, autophagosomal fusion with lysosomes was suppressed in *PRIP*-KO cells in lysosome staining experiment.

CONCLUSION : These data suggested that the deletion of PRIP in MEFs induces prevention of the autolysosome formation, and *S. aureus* could proliferate more easily in *PRIP*-KO cells than in wild type cells. All together, we concluded that PRIP modulates the autophagy pathway caused by bacterial invasion.

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18-19

Post-translational modification of integrin β8 by ubiquitin-proteasome system in oral squamous cell carcinoma cell lines

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BACKGROUND : Integrins were heterodimeric transmembrane receptors for extracellular matrix proteins. We have shown that the interaction of αvβ8 and type I collagen promotes the proliferation and migration of oral squamous cell carcinoma (SCC) cells.

OBJECTIVES : Some oral SCC cell lines have expressed little amount of β8 protein in spite of the expression of β8 mRNA, suggesting the post-translational modification of β8 protein.

In present study, we examined the role of ubiquitin-proteasome system in post-translational modification of integrin β8 in SCC cell lines.

EXPERIMENTAL METHODS : The expression of β8 protein in oral SCC cell line, SCCKN treated with proteasome inhibitor was examined by immunoblotting. To detect the interaction between β8 and human double minute 2 (Hdm2), co-immunoprecipitation and mammalian two-hybrid assay were performed.

Human vulval carcinoma cell line, A431, which expresses little amount of mRNAs of αv and β8, was transfected with αv gene, and A431αv was isolated. To induce β8 protein temporarily, A431αv cells were transfected with tetracycline inducible expression vector encoding β8 gene. Alteration of β8 protein induced by temporary treatment with tetracycline in A431mock and A431αv was examined.

RESULTS : Treatment of oral SCC cells with proteasome inhibitor and Hdm2 E3 ligase inhibitor led to the enhancement of expression of β8 protein. Co-immunoprecipitation and mammalian two-hybrid assay indicated that β8 formed a complex with Hdm2.

Treatment of tetracycline for 24h induced the expression of β8 protein in both A431mock and A431αv. Sequential cultivation in the absence of tetracycline led to almost complete loss of β8 protein in A431mock. In contrast, the cultivation without tetracycline had no influence on the expression of β8 protein in A431αv.

CONCLUSION : Monomeric β8 is ubiquitinated by Hdm2, and degradated by proteasome in SCC cells. In contrast, β8 dimerized with αv is stable compared to β8 monomer by the escape from ubiquitin-proteasome system.

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18-21

Participation of heterodimer formation with integrin αv subunit in the stability of integrin β6 subunit in squamous cell carcinoma cells

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INTRODUCTION : Integrins were heterodimeric transmembrane receptors for extracellular matrix (ECM) proteins, which are consisted with one α subunit and one β subunit. Formation of the α/β heterodimer is essential for the expression and the function of integrins on cell surfaces.

Our previous study has shown that the expression of integrin β6 subunit is regulated by ubiquitin-proteasome system in some oral squamous cell carcinoma (SCC) cells. In present study, we examined the effect of the formation of α/β heterodimer on the stability of β6 subunit in SCC cells.

EXPERIMENTAL PROCEDURES : Integrin αv gene was subcloned into mammalian expression vector pCI-neo, and the resultant plasmid was termed as pCI/neo-αv. Human vulval SCC cell line A431, which expresses little amount of mRNAs of integrin αv and β6, was transfected with pCI/neo or pCI/neo-αv, and A431mock or A431αv were isolated, respectively. Next A431mock and A431αv were transfected with a tetracycline (Tet) inducible expression vector pCDNA4/TO containing β6 gene, and A431mock/β6-On and A431αv/β6-On were isolated. Altered expression of β6 protein induced by tetracycline was examined in A431mock/β6-On and A431αv/β6-On.

RESULTS : Treatment with Tet induced the expression of β6 protein in both A431mock/β6-On and A431αv/β6-On. Sequential cultivation in the absence of Tet led to a gradual decrease in the expression of β6 protein in A431mock/β6-On, and almost complete loss of β6 protein was observed after 12h. In contrast, the cultivation without Tet has no influence on the expression of β6 protein in A431αv/β6-On.

CONCLUSION : β6 subunits induced by Tet in A431mock/β6-On might exist as a monomer because of the insufficiency of αv subunits available for forming αvβ6 heterodimer. In contrast, β6 subunit induced by tetracycline in A431αv/β6-On dimerizes with abundantly expressed αv subunit. The possibility should be considered that β6 dimerizes with αv is stable compare to β6 monomer by the escape from ubiquitin-proteasome system.

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18-22

Snail-dependent upregulation Gal-1 promoted to complete EMT process in Snail Expressing cells

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OBJECTIVE : Epithelial-mesenchymal transition (EMT) is a complex and reversible biological process, where an epithelial tumor cell alters its polarized and homophilic phenotype to the mesenchymal phenotype (e.g., single cell migration and invasion). By microarray analysis using SCC cells with or without EMT phenotype and Snail-induced EMT cells, we identified a cluster of transcripts of which expression is controlled by EMT. From this gene clustering we found that Galectin 1 (Gal-1) was highly upregulated in EMT phenotype cells. Gal-1 is a member of the β-galactoside-binding lectin family protein. We characterized how Gal-1 participates SCC cells behaviors in vitro.

MATERIAL AND METHODS : We generated Gal-1 expressing squamous cell carcinoma cells (A431 and OM-1) to test their behaviors in matrigel invasion assay and wound healing assay.

RESULTS : Gal-1 overexpression and recombinant Gal-1 treatment accelerated collective cell migration and invasion. We found that Gal-1 activated Rac to promote filopodia. Notably, integrin α2 and β5 expression were induced in Gal-1 overexpressed cells. By functional attenuation either to integrin α2 and integrin β5, the invasiveness was suppressed as well as by functional Gal-1 blocking with anti-Gal-1 antibody. Moreover, recombinant Gal-1 increased the numbers of EMT cells in Snail-expressing SCC cells in growing condition (24% to 42%).

CONCLUSION : These results suggested that upregulation of integrin α2 and β5 were involved in Gal-1 dependent invasiveness of homophilic SCC cells. Elevating paracrine Gal-1 alone did not induce EMT, but Snail-expressing SCC cells increased susceptibility to undergo EMT by Gal-1.

Key words : EMT, Snail, Galectin-1

B-26

18-23

Extracellular inorganic polyphosphate decreases inducible nitric oxide synthase expression and nitric oxide production induced by lipopolysaccharide in macrophages

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BACKGROUND AND OBJECTIVE : Inorganic polyphosphate [poly(P)], a linear polymer composed of tens to hundreds of orthophosphates residues, widely found in organisms ranging from bacteria to mammals. Recent studies have reported that extracellular poly(P), which is released from cells, plays important roles in bone remodeling, blood coagulation, and inflammation. However, despite the ubiquitous distribution of poly(P) in mammalian tissues and cells, current knowledge pertaining to the functions of poly(P) is limited. This prompted us to further investigate the functions of poly(P). In this study, we examined whether extracellular poly(P) could affect macrophage responses to lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria.

METHODS : Male C57BL/6J mice (8-12 weeks old) were intraperitoneally injected with thioglycollate medium. After 3 days, peritoneal exudate macrophages were harvested and were then treated with poly(P) and LPS. Inducible nitric oxide synthase (iNOS) mRNA and protein were analyzed by real-time PCR and Western blotting, respectively. Nitric oxide (NO) was determined by the Griess reaction. Tumor necrosis factor (TNF) was measured by ELISA. Cell viability was assessed using the WST-8 assay and trypan blue exclusion assay.

RESULTS AND CONCLUSION : In mouse peritoneal macrophages, poly(P) significantly suppressed LPS-induced expression of iNOS, which is essential for host defense against infection, without affecting cell viability. Poly(P) with longer chains is more potent in suppressing iNOS expression than that with shorter chains. Poly(P) decreased LPS-induced NO release as well as iNOS expression. In addition, poly(P) suppressed iNOS mRNA expression induced by LPS, indicating that poly(P) reduces iNOS expression by down-regulation at the mRNA level. In contrast, poly(P) did not affect the LPS-induced release of TNF, an inflammatory cytokine. These results suggest that extracellular poly(P) serves as a regulatory factor of innate immunity by modulating iNOS expression in macrophages.

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18-26

The FGFR-1 inhibitor PD173074 induces mesenchymal-epithelial transition through the transcription factor AP-1

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BACKGROUND : Epithelial-mesenchymal transition (EMT) is a crucial process in cancer progression that provides cancer cells with the ability to escape from the primary focus, invade stromal tissues and migrate to distant regions. Cell lines that lack E-cadherin show increased tumorigenesis and metastasis, and the expression levels of E-cadherin and Snail correlate inversely with the prognosis of patients suffering from breast cancer or oral squamous cell carcinoma (OSCC). Moreover, recent studies have shown that most EMT cases are regulated by soluble growth factors or cytokines. Among these factors, fibroblast growth factors (FGFs) execute diverse functions by binding to and activating members of the FGF receptor (FGFR) family, including FGFR1 - 4. FGFR1 is an oncoprotein that is involved in tumorigenesis, and PD173074 is known to be a selective inhibitor of FGFR1. However, the roles of FGFR1 and FGFR1 inhibitors have not yet been examined in detail.

METHODS : Here, we investigated the expression of FGFR1 in head and neck squamous cell carcinoma (HNSCC) and the role of the FGFR1 inhibitor PD173074 in carcinogenesis and the EMT process.

RESULTS : FGFR1 was highly expressed in 54% of HNSCC cases and was significantly correlated with malignant behaviours. Nuclear FGFR1 expression was also observed and correlated well with histological differentiation, the pattern of invasion and abundant nuclear polymorphism. FGFR1 was also over-expressed in EMT cell lines compared to non-EMT cell lines. Furthermore, treatment of HOC313 cells with PD173074 suppressed cellular proliferation and invasion and reduced ERK1/2 and p38 activation. These cells also demonstrated morphological changes, transforming from spindle- to cobble stone-like in shape. In addition, the expression levels of certain matrix metalloproteinases (MMPs), whose genes contain activator protein 1 (AP-1) promoter sites, as well as Snail1 and Snail2 were reduced following PD173074 treatment.

CONCLUSION : Taken together, these data suggest that PD173074 inhibits the MAPK pathway, which regulates the activity of AP-1 and induces mesenchymal-epithelial transition (MET). Furthermore, this induction of MET likely suppresses cancer cell growth and invasion.

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18-27

Tumor suppressive role of Ameloblastin through Src inactivation in osteosarcoma

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BACK GROUND : Ameloblastin (AMBN), the most abundant non-amelogenin enamel matrix protein, has a role in ameloblast differentiation. Moreover, we clarified that AMBN induces osteogenic differentiation through AMBN-CD63-Integrin β1-Src axis (Mol Cell Biol. 2011). During this experiment, we found that AMBN-overexpressing osteosarcoma cells showed suppression of growth and migration. Osteosarcoma occurs in long bones and jaws and still shows a poor prognosis in association with lung metastasis, despite the advances in chemotherapy.

OBJECTIVES : The aim of this study was to demonstrate that AMBN has a novel tumor suppressive role in osteosarcoma.

EXPERIMENTAL METHODS : Osteosarcoma cell lines; NOS-1, SaOS-2, MG-63, HOS, 143B-Luc cells were used. For *in vitro* study, tumor growth analysis, wound healing assay, RT-PCR, western blot and immunofluorescent analysis were used. For *in vivo* mouse xenograft model, 5-week-old nude mice were analyzed by *in vivo* imaging assay using luciferase. Clinical data of 37 osteosarcoma cases were used for the immunohistochemical analysis. Statistical analyses were conducted using Student's *t*-test, chi-squared test, or log-rank test. $P < 0.05$ was considered significant.

RESULTS : (1) In immunohistochemical analysis of 37 clinical osteosarcoma cases, reduced expression of AMBN was observed in the cases with lung metastasis and poor prognosis. (2) *In vivo* mouse xenograft model, AMBN inhibited tumor growth and lung metastases of osteosarcoma. (3) *In vitro*, AMBN expression was negatively correlated with c-myc expression among osteosarcoma cell lines, and ectopic overexpression of AMBN suppressed proliferation through c-myc via Src inactivity in SaOS-2 cells. Furthermore, AMBN overexpression suppressed migration through the formation of stress-fiber and focal adhesions through RhoA activation via Src inactivity in SaOS-2 cells.

CONCLUSION : We demonstrated that AMBN had a tumor suppressive role via Src inactivation in osteosarcoma. Our findings indicate that AMBN can be a new prognostic marker and therapeutic target for osteosarcoma. This study was supported by JSPS.

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18-28

Identification of microRNA-203 as an inhibitor of invasion in oral cancer

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BACK GROUND : microRNAs (miRNAs) are highly conserved small non-coding RNAs that downregulate specific target expressions through suppressing translation or inducing degradation of mRNAs. Recent cumulative evidence have been shown that microRNAs play important roles in the invasion and metastasis of various cancers.

OBJECTIVE : This study was designed to identify miRNAs involved in the invasion of oral cancer.

EXPERIMENTAL METHODS : We compared the gene expression profiles between MSCC-inv1, a highly invasive clone and MSCC1 cells, parent oral squamous cell carcinoma (OSCC) cell line. Thereby, we focused on miR-203 because it was markedly downregulated in MSCC-inv1 cells and examined the role of miR-203 by using OSCC cell lines. Furthermore, to clarify the downstream of miR-203, we compared the mRNA binding to Argonauto(Ago)2, which form complex with miRNA and its target mRNA, with control and miR-203 overexpressing cells by microarray analysis.

RESULTS : Several miRNAs including miR-203 were downregulated in MSCC-inv1 cells and Epithelial-mesenchymal transition (EMT) induced OSCC cell lines. Intriguingly, overexpression of miR-203 suppressed cell invasion while inhibition of miR-203 promoted cell invasion inversely. Moreover, we identified Gene X as a novel target of miR-203. Gene X was reduced by miR-203 overexpression and elevated by miR-203 inhibitor. Interestingly, we found Gene X was upregulated in TGF-β induced EMT process. Additionally, overexpression of miR-203 suppressed upregulation of Gene X and EMT induction. Importantly, immunohistochemical study showed that high expression of Gene X correlated with aggressive pattern of invasion and regional metastasis.

CONCLUSIONS : miR-203 is involved in EMT process through Gene X regulation and suppresses invasion. Reduced miR-203 may be critical event for invasion and metastasis in oral cancer.

B-30

18-29

Effect of F-spondin on LPS-induced periodontal inflammation and bone destruction

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BACKGROUND : We previously reported that F-spondin plays an important role in the differentiation of cementoblasts (BBRC, 2006 and 2012). Moreover, we recently revealed that F-spondin expression in human cementoblast-like cell lines (HCEM) increases by stimulation of *Aggregatibacter actinomycetemcomitans*-lipopolysaccharide (LPS). However, it is still unclearly known about the role of F-spondin in periodontitis.

OBJECTIVE : Present study was designed to clarify the role of F-spondin in LPS-induced periodontal inflammation.

METHODS : We used immortalized HCEM, human periodontal ligament cell lines (HPL) and HPL-SPON1 transfected with a rat F-spondin cDNA into HPL. F-spondin expression of HCEM was knock-downed by siSPON1. To investigate the effect of F-spondin on inflammatory responses induced by LPS in these cells, we examined expression of inflammatory cytokines mRNAs by RT-PCR and LPS related signaling pathway by western blot. Furthermore, we examined inflammatory reactions of periodontal tissues induced by LPS in F-spondin transgenic mice (SPON1-TG) in comparison with those in wild type mice (WT).

RESULTS : LPS-treatment down-regulated IL-6 expression in HCEM. Expression of TNF-α, IL-1β and IL-8 did not show remarkable change between with or without LPS treatment. siSPON1 recovered down-regulation of IL-6 induced by LPS in HCEM. On the other hand, F-spondin overexpression significantly decreased IL-6 expression. Moreover, phosphorylation of Erk induced by LPS was detected in HPL, but not in HPL-SPON1. Moreover, *in vivo*, the number of mature osteoclasts and neutrophils induced by LPS in the periodontal tissue were fewer in SPON1-TG than in WT. SPON1-TG showed less bone destruction than WT, but there is no significant difference in cementum resorption.

CONCLUSION : F-spondin plays anti-inflammatory role in periodontitis by down-regulation of IL-6 expression through inhibiting activation of Erk pathway. Therefore, F-spondin in the cementoblasts may protect cementum from pathologic resorption. Moreover, F-spondin might be a novel applicable molecule for prevention of inflammation and bone destruction associated with periodontitis.

B-31
18-30

The importance of VEGF-Flt-1 signaling in oral cancer progression

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INTRODUCTION : Vascular endothelial growth factor (VEGF)-mediated angiogenesis plays a critical role in tumor growth and metastasis. VEGF binds two tyrosine kinase receptors (Flt-1 and Flk-1) and stimulates endothelial cell mitogenesis and migration. It is reported that the VEGF-Flt-1 signaling may contribute to cell migration of macrophages, osteoclasts and tumor cells including leukemic and melanoma cells. However, the direct role of VEGF-Flt-1 signaling on oral squamous cell carcinoma (OSCC) cells growth and invasiveness is not well understood. The aim of the present study is to clarify the direct effects of VEGF-Flt-1 signaling on OSCC cells.

MATERIAL AND METHODS : We investigated mRNA and protein expression of VEGF and Flt-1 in 6 OSCC cell lines. Then HSC2 (high Flt-1 expression cell line) and HO1-N1 (no Flt-1 expression cell line) were used in this study. The cell number was counted with 0-10 ng/ml of Placental growth factor (PlGF; a Flt-1 specific ligand). The effects of PlGF (1-100ng/ml) on migration and invasion were examined using Boyden chamber method with or without matrigel. The PlGF induced MMPs-expression and its related pathway were also examined.

RESULTS : OSCC cells except for HO1-N1 expressed Flt-1. Flt-1 activation by low concentration of PlGF stimulated the proliferation of HSC-2. Migration and invasion of HSC2 were also promoted by PlGF treatment. However, PlGF treatment showed no effect on proliferation, migration and invasion in HO1-N1. The expression level of MMP9 is markedly up-regulated by PlGF treatment. Moreover, PlGF induced the phosphorylation of p38 and ERK1/2 in HSC2. Inhibition of ERK1/2 activation inhibited the up-regulation of MMP9 mRNA expression induced by PlGF.

CONCLUSION : VEGF-Flt-1 signaling plays an important role in OSCC progression by facilitating invasion and migration of OSCC through ERK1/2-MMP9 pathway. The blocking of VEGF-Flt-1 signaling may be beneficial for the treatment of OSCC patients.

B-32

18-31

Pathological progression of non-alcoholic steatohepatitis is exacerbated by dental infection of *Porphyromonas gingivalis*

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INTRODUCTION : Non-alcoholic steatohepatitis (NASH) is a liver phenotype of metabolic syndrome. 10-25% of NASH progresses to lethal diseases of cirrhosis and liver cancer. Recently, it is reported that odontogenic infection may worsen a metabolic syndrome like type 2 diabetes. However, its effect on NASH is unclear. We reported that the odontogenic infection of *Porphyromonas gingivalis (P.g.)* deteriorated the progression of NASH and suggested that *P.g.* or *P.g.* derived-LPS (*P.g.*-LPS) from the lesion of odontogenic infection can lead to exacerbation of inflammation, fibrosis and lipid deposition in NASH. In the present study, to clarify the importance of dental therapy for prevention or treatment of NASH, I examined the mechanism for pathological progression of NASH by *P.g.* and *P.g.*-LPS.

MATERIAL AND METHODS : Palmitate pretreated human hepatocytes were used as a steatotic hepatocyte model. For analysis of *P.g.* infection, the cells were reacted with *P.g.* at a multiplicity of infection of 100 for 2 hrs. The cytokines, TLRs, integrin and lipid uptake receptors expression in hepatocytes with/without *P.g.*-LPS stimulation or *P.g.* infection was examined by RT-PCR. RT-PCR of *P.g. mgl* gene and antibiotic protection assay were performed.

RESULTS : 1) TLR2 expression is upregulated in steatotic hepatocytes. The activation of TLR2 pathway by *P.g.*-LPS can induce excessive production of cytokines which eventually leads to exacerbation of inflammation and fibrosis. 2) *P.g.* can easily invade into steatotic hepatocytes through the upregulation of integrin. 3) *P.g.* infection may induce the upregulation of mRNA expression of LDLR and LRP1 (lipid uptake receptors) resulting in the increase of lipid deposition in hepatocytes.

CONCLUSION : The vicious cycle of NASH progression by *P.g.*-odontogenic infection is established in liver. Prevention and/or elimination of *P.g.* infection by dental therapy may have a beneficial impact on NASH.

C-1
05-1

Corrosion Properties of the Stainless Steel Bracket

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BACKGROUND : Stainless steel orthodontic brackets have been used most frequently for fixed orthodontic treatment. The different between each product shows that many variation of the composition material. Tis condition influence the corrosion properties of the bracket.

OBJECTIVE : Present study was designed to investigate the corrosion properties of the commersial bracket.

EXPERIMENTAL METHODS : To evaluate the corrosion properties of the brackets in an oral environment, potentiodynamic testing was performed in artificial saliva (Table 3) at 37 ± 1˚C. The exposed area of the samples to the solution was 1 cm^2. A saturated calomel electrode was used as the reference electrode. The cathodic polarization was performed to a certain potential below the open circuit potential to eliminate the scale. The specimens were stabilized at an open circuit potential for 5 minutes. The potential scan was started from the corrosion potential at a scan rate of 50 mV/minute. To evaluate the corrosion potential, polarization resistance and corrosion rate of the samples according to ASTM designations G3 and G102, the linear polarization and Tafel extrapolation techniques were used. The corrosion potential and polarization resistance were obtained from the linear polarization and corrosion rate, as calculated using the Stern-Geary equation, after measuring the anodic and cathodic Tafel slopes from the polarization curves.

CONCLUSSION REVIEW : Although corrosion of orthodontic devices occurs, it does not appear to result in significant destruction of the metallic components or have significant detrimental effects on mechanical properties. Exceptions to this might be soldered joints on the brazed joints of some stainless steel brackets.

C-2
06-1

Effect of bleaching on surface roughness and surface topography of veneering materials- an atomic force microscope study

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BACKGROUND : Bleaching occurs when unstable free radicals alter the chemical structure of organic substances within teeth. Since bleaching agent is held in intimate contact with teeth and associated restorations, there remain concerns regarding the possible effects on dental restorations. One such effect found was increased surface roughness, which could detrimentally affect longevity of restorations.

OBJECTIVE : To assess *in vitro* the surface roughness and surface topography of microhybrid composite resin and feldspathic porcelain veneering materials following exposure to 20%carbamide peroxide and 40%hydrogen peroxide bleaching agents.

MATERIALS AND METHODS : Standardized cylindrical specimens of microhybrid composite resin were prepared using Ceramage®. Feldspathic porcelain specimens were made uniform by sectioning of CAD/CAM Cerec® blocs. Negative control groups comprising of two specimens from each substrate were subjected to baseline surface roughness (Ra, nm) and topography assessment using an atomic force microscope. Specimens from each substrate were then randomly divided into two subgroups (n=4), receiving different surface treatments: 20%carbamide peroxide and 40%hydrogen peroxide. The 20%agent was applied 4hours daily for 7days and the 40%agent was applied in three 20-minute cycles, at 37˚C respectively. Thereafter, surface roughness and surface topography of all the specimens were evaluated using the atomic force microscope. Surface topography images were subjectively assessed and surface roughness data were analyzed using Kruskal-Wallis and Mann-Whitney U tests at 5% significance level.

RESULTS : For both substrates, there were no statistically significant differences detected between the control and bleached groups (p=0.878). Surface topography images reveal that bleached surfaces were qualitatively rougher compared to the control groups, and the higher the concentration of peroxide, the rougher the surface. However, changes in roughness were minimal and were not statistically or clinically significant.

CONCLUSION : Surface roughness of the evaluated microhybrid composite resin and feldspathic porcelain materials was not detrimentally affected by bleaching with 20%carbamide peroxide or 40%hydrogen peroxide.

C-3
09-1

Curing depth of bulk fill resin composites

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BACKGROUND : With advance technology, bulk fill resin composite was introduced to simplify filling technique as they are claimed to be cured in single increment at 4-6 mm from top surface. Thus, adequate light energy transmission is essential to obtain optimum mechanical properties.

OBJECTIVES : To evaluate and compare curing depth of bulk fill resin composite available in Thailand using ISO 4049 method and Vickers hardness bottom-to-top ratio.

METHOD : Three bulk fill materials (SonicFill [SF], Tetric N-Ceram Bulk fill [TB], X-tra Fill [XF]) and one control material (Z350 XT) were prepared in cylindrical stainless steel molds and irradiate with LED light source (1,100mw/cm^2) for either 10 or 20 seconds (n = 10 per group). Depth of cure was determined according to "ISO 4049; Depth of cure" method. After storing in distilled water for 24 hours at 37°C, all samples were cut down to curing depth recommended by manufacturer. Bottom-to-top hardness ratio was measured using 200g of Vickers diamond indentation for 15 seconds. Then, all samples were cut down 0.5 mm at a time to define depth which represented 80% bottom-to-top hardness ratio. Cure and hardness were analyzed with one-way ANOVA followed by Tukey HSD post-hoc test.

RESULTS : XF significantly exhibited highest depth of cure for both 10s and 20s irradiation time (4.53 ± 0.13 mm and 5.09 ± 0.2 mm respectively). When applied 20 seconds of LED light curing, XF and TB had a significant increase of curing depth. For hardness measurement, the acceptable 80% ratio was found at 4 mm depth for XP while TB and SF were met at 3 mm depth with 20 seconds of curing time.

CONCLUSION : From this study, all materials met with ISO standard except TB with 10 seconds exposure time. Depth of cure and hardness were increased by increasing irradiation time. Only 20 seconds of irradiation time exhibited sufficient bottom-to-top hardness ratio at 3-4 mm depth.

C-4
13-1

The protective effect of albumin corona on nanoparticles

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BACKGROUND : With the rapid development of nanotechnology in the past few decades, many attentions have been paid to the application of nanoparticles (NPs) in drug or imaging agent delivery. Unfortunately, the successful delivery of NPs is limited by the formation of proteins corona surrounding NPs immediately after their entry into the blood. The non-specifically adsorbed plasma proteins will cover the original surface properties of NPs and become the real substance that the body organs and cells firstly "see". This is the main reason for the rapid clearance of NPs from the blood following intravenous injection.

OBJECTIVES : In this present work, we aim to investigate the impacts of the preformed albumin corona around NPs (NPs-BSA complex) on the physicochemical and biological properties of NPs, and evaluate its ability in extending the circulation time of NPs.

METHODS : NPs-BSA complex, formed by incubating NPs with BSA solution, was confirmed by size, zeta potential and morphology changes. The impacts of BSA corona on the plasma proteins adsorption, macrophages uptake, and cytotoxicity of NPs were also studied. The pharmacokinetics study for the original NPs and NPs-BSA was performed in rats.

RESULTS : The stable NPs-BSA complex could be formed after incubation at room temperature for 2 h. After intravenous administration, the half-life of NPs-BSA was significantly longer than that of the original NPs, probably due to the inhibited plasma protein adsorption and the reduced macrophages uptake in the presence of BSA corona. Meanwhile, the cytotoxicity of NPs was also significantly reduced by formation of NPs-BSA complex.

CONCLUSION : Our findings suggest that formation of NPs-albumin complex is effective and feasible to prolong the NPs circulation time and reduce its toxicity. It has a great potential to be a versatile strategy for optimizing the NPs based drug delivery systems.

C-5
15-2

Effects of histomorphometric, bone-to-implant contact and osseointegration on a novel hybrid micro/nano topography-modified dental implant in the mandibular canine-premolar area of the mini-pigs

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BACKGROUND : Electrochemical oxidation following a sandblasted and acid-etched (SLA-Ti) treatment has gained much interest as a surface modification for titanium (Ti) implants (SLAffinity-Ti); however, less information is available on the impact for *in vivo* performances of these SLAffinity-Ti implants.

OBJECTIVES : The present study is to evaluate the osseointegration and biomechanical bone tissue response to SLAffinity-Ti implants possession of micro-and nanoporous oxide layers.

EXPERIMENTAL METHODS : Seventy-two implants belonging to the following groups (12 of each group): a standard machined-Ti (M-Ti) surface, a SLA-Ti surface and a SLAffinity-Ti surface were inserted into the mandibular canine-premolar area of mini-pigs. The histomorphometric and removal torque tests were conducted after 3 and 12 weeks of implantation.

RESULTS : The implants with the SLAffinity-Ti surface caused more peri-implant bone density and bone-to-implant contact than those with the SLA surface. Electrochemical oxidation both increased the torque resistance to removal of SLAffinity-Ti implants. The difference was statistically significant ($p<0.001$) after 3 weeks of implantation, whereas no statistical difference was observed after 12 weeks of implantation ($p>0.005$).

CONCLUSION : After 3 weeks of healing, the bone microstructure around SLAffinity-Ti implants appeared significantly more organized, achieving a higher stability in bone. Clinical implications of these results included an early peri-implant formation of bone and an indication for earlier loading protocols.

C-6
15-4

Evaluation of patient characteristics as potential prognostic factors for dental implant failures by using classification and regression tree analysis

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BACKGROUND : Dental implant assessment technique has been widely applied nowadays. However, a great number of failure rate cases have been investigated and reported continuously. Despite the fact that clinic implant systems are more invasive and costly than traditional prosthodontics, these often cause medical disputes in Taiwan's healthcare systems.

OBJECTIVES : The aim of the study is to analyze the risk factors related to patient characteristics and implant therapy, thereby giving some surgical guidelines in avoiding such failures by clinical dentists.

EXPERIMENTAL METHODS : We used classification and regression tree (CART) to establish the relationship between the important independent variables and target variable. The study collected during July, 2003 to August, 2010. These implant cases were obtained from three clinics in new Taipei city for failure analysis of implants after implantation. It covers 531 cases, involving 1,189 implants in total, gathering relevant information on basic demographic factors, health status, living style, oral hygienic habits, anatomic feature, implant attributes, denture attributes.

RESULTS : The average age of the individuals in the cases was 47.5, and the success rate was 91.3%. Based on the results of CART, it was found that the important variables are bone density, alveolar ridge morphology, biting of hard objects, and the quality of bone-grafting materials. The failure rate varies between 2.4% to 58.1% and it is clearly influenced by the biting of hard objects or bone-grafting materials.

CONCLUSION : According to potential prognostic factors, two ways to decrease the risk of implant failure in the cases of bone defect: one is to postpone the time of implant until the amount of defected bone shape and volume raised and second, is to graft as much patients' autogenous bone powder as possible during implantation. However, more patient cases should be collected and analyzed to offer a valuable guidance for dentists practice in the future.

C-7
18-6

Influence of micro mechanical retention with diode laser beam machine on the bond strength of porcelain fused to zirconia

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BACKGROUND : In recent years, zirconia-supported ceramic crown have been used widely in association with advancement of CAD/CAM systems. Our hypothesis is that fabricating the retention on zirconia demonstrates the high bond strength with veneering porcelain. However, it is difficult for milling systems to fabricate some micro retention on the zirconia coping for support veneering ceramics because sizes of bur have limitations.

OBJECTIVES : The aim of this study was to examine the influence of micro retention with laser beam machine on the bond strength between zirconia (Y-TZP, NANO ZR) and veneering porcelain.

MATERIALS AND METHODS : Two types of zirconia (Y-TZP and NANOZR) were used in this study. Two surface treatments, alumina blasting with 125 μm alumina particles (AB), fabricating the micro retention with diode laser beam machine (MR), were performed to zirconia specimens. In addition, non-treated specimens (NT) were used as a control. After veneering porcelain fused to zirconia specimens, shear bond strengths were measured.

RESUITS : In the Y-TZP, the mean bond strength ranged from a maximum of 25.7 MPa (MR group) to a minimum of 23.4 MPa (NT group). In the NANOZR, bond strength ranged from a maximum of 25.7 MPa (MR group) to a minimum of 20.5 MPa (AB group). However, no statistical significant differences were found.

CONCLUSION : Micro mechanical retention with diode laser beam machine has no effect on the bond strength of porcelain fused to zirconia.

C-8
18-14

Evaluation of proliferation and differentiation of messencymal stem cells on mixed self-assmebled monolayers.

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OBJECTIVE : Self assnembeld monolayers (SAMs) give well-defined model surfaces for studies on interfacial phenomena and intermolecular interactions. We made mixed SAMs with various ratios of amino, hydroxyl, carboxyl, and methyl groups and report the relationship between the surface compositions and water contact angles, as the surface composition change induces cell proliferation of the growth pattern. We report the different of messencymal stem cells (MSCs) proliferation and differentiation.

METHODS : 1 mmol/L of amino, hydroxyl, carboxyl, and methyl terminated alkanethiol solutions were prepared in ethanol. These alkanethiol solutions were mixed at various ratios. The gold plates were immersed in these solutions, and the mixed SAMs were formed on the gold substrates.

MSCs were cultured on the mixed SAMs for 3 days in a medium containing 10% FCS and in a serum-free medium (STK2).

Chondrogenic and adipogenic differentiaion of MSCs were performed on a corning cell culture dish and the mixed SAM in differentiation-inducing culture mediums. Differentating cells were stained and observed using a microscope.

RESULTS : We evaluated the patterns between surface composition and cell proliferation with and without serum. Various kinds of proteins are contained in serum but their concentrations are not completely defined and nonconstant quality in each lot of serums, whereas STK2 is well-difined solution. On mixed SAMs, the patterns of cell proliferation were quite different between the two cases, and cell proliferation was more noticable in STK2 than serum contained medium.

In the condrogenic and adipogenic differention, the mixed SAM enhanced differentiation of MSC compared with the cell culture dish.

CONCLUSION : We firstly developed the completely well-defined cell culture system by the chemically well-defined combination of culture medium and plate. This achievement will be evolved the field of cell biology and regenerative medicine at a rapid pace.

C-9
18-20

Generation and serial cultivation of induced pluripotent stem cells from dental pulp cells in serum-free and feeder-free defined culture

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BACK GROUND : Human Embryonic Stem (hES) cells and human induced Pluripotent Stem (hiPS) cells are commonly maintained on inactivated mouse embryonic fibroblast feeders (MEF) in fetal bovine serum- or KSR-supplemented medium. However, one of the major obstacles to such uses for hiPS cells is the risk of contamination from undefined pathogens in conventional culture conditions that use serum replacement and MEF. Furthermore there is no consensus as to the optimal formulation, or the nature of the cytokine requirements of hiPS cells to promote their self-renewal and inhibit their differentiation. Previously, we have developed a growth factor-defined serum-free medium designated hESF9, for the culture of human ES cells. This medium permits their prolonged culture in an undifferentiated state without feeder cells.

OBJECTIVES : This study aims to generate hiPS cells from dental pulp cells in serum-free and feeder-free defined culture condition to elucidate the nature of the cytokine requirements of the cells to promote their self-renewal and inhibit their differentiation.

EXPERIMENTAL METHODS AND RESULTS : We tried to generate hiPS cells from dental pulp cells with four factors: Oct3/4, Sox2, KLF-4 and c-Myc in hESF9 serum-free medium. Eighteen days after transfection, the hES-like cell colonies have been formed on fibronectin (FN) -coated dish in hESF9 medium. We picked the colonies up and continued cultivating in hESF9 on FN-coated dishes. These colonies possessed ES cell-like morphology, proliferation activities, surface markers, gene expression, and differentiation activities into cell types of the three germ layers by virtue of embryoid body formation in vitro and teratoma formation assay in vivo. Currently we have serially subcultured the cells in an undifferentiated state with more than 70 passages under serum- and feeder-free culture condition.

CONCLUSION : We have succeed to generate hiPS from adult human dental pulp cells and maintained in an undifferentiated state in serum-free and feeder-free defined medium.

C-10
18-3

Generation of disease-specific human induced pluripotent stem (iPS) cells from dental pulp cells of a patient with Cleidocranial dysplasia in serum- and feeder-free culture.

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BACKGROUND : In the field of genetic diseases, iPS cells have become an appealing choice for elucidate pathogenesis and treatments. In this study, we generated disease-specific iPS cells derived from dental pulp cells of a patient with Cleidocranial dysplasia (CCD). CCD is an autosomal dominant inherited skeletal disease caused by mutations in Runx2 which shows the abnormal differentiation of bone and cartilage.

METHODS AND RESULTS : We have successfully generated disease-specific human iPS cells from dental pulp cells of a CCD patient using Yamanaka's factors (Oct3/4, Sox2, Klf4 and c-Myc) with retroviral vectors in serum- and feeder-free defined medium on fibronectin-coated culture condition. CCD-iPS cells retained the property of self-renewal and have an undifferentiated phenotype by virtue of the expression of Oct3/4, Nanog, Sox2, Esg1, Rex-1 and alkaline phosphatase (ALP). Furthermore, we found that CCD-iPS cells express several human embryonic stem cell marker proteins. It has been confirmed that CCD-iPS cells could differentiate into all three germ layers in embryoid body formation assay in vitro and teratoma formation in vivo. To identify the differentiation ability of CCD-iPS cells, we evaluated their osteogenic and chondrogenic differentiation activities. It has been revealed that ALP activity exhibited a striking impairment in osteogenic differentiation of CCD-iPS derived cells compared to that of wild-type human iPS derived cells. Difference in chondrogenic activity was further confirmed by Alcian Blue staining and proteoglycans synthesis. This impairment was further supported by real-time PCR analysis of chondrogenic marker genes such as RUNX2, collagen2A1, and collagen10A1. In addition, we normalized the gene mutation of CCD-iPS using transcription activator-like effector nucleases (TALEN) which drive targeted gene modifications, and performed a functional analysis.

CONCLUSIONS : We have successfully established disease specific iPS cells from CCD patient. Further characterization of CCD-iPS cells would be beneficial to clarify the molecular mechanism involved in the disease.

D-1
01-3

Nigella sativa oral rinse as an anti oxidant effect reduced bleeding on probing and pocket depth

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BACKGROUND : Nigella sativa extract have been shown to suppress the growth of supragingival and subgingival bacteria. Nigella sativa extract were reported as an anti inflammation. The beneficial effects of nigella sative extract were shown to be related to a reduction of the inhibition of inducible nitric oxide synthase and interleukin (IL)-1β expression. The rationale of the study is based on our previous studies demonstrating the beneficial antioxidant effect of nigella sativa extract 3 % in vitro.

PURPOSE : The aim of the present study is to assess the clinical efficacy of nigella sativa oral rinse 3 % in managing of symptoms associated with progressivity of chronic periodontitis as a bleeding on probing and pocket depth.

MATERIALS AND METHODS : Patients with chronic periodontitis will randomly receive nigella sativa extract oral rinses. Thirty adult chronic periodontitis patient divided into two groups. group 1: comprised fifteen chronic periodontitis involved sites managed by scaling root planing alone. And group II: comprised fifteen chronic periodontitis involved sites treated by the same technique in adjunct with the application of antioxidant mouth rinse nigella sative extract 3 %. The patients will rinse 5 ml of the mouthwash, 2 times daily for 14 days. Clinical examination include probing depth (PD) and bleeding on probing (BOP).

RESULTS : These findings suggest that nigella sativa oral rinse 3% may actually decrease bleeding on probing dan pocket depth significantly ($p<0,05$).

CONCLUSION : We report that nigella sativa oral rinse provides inhibitor effect against bleeding on probing and pocket depth. Nigella sativa oral rinse 3% as an antioxidant effect potential as adjunct theurapetic agent after scaling root planing to prevent progressivity of chronic periodontitis.

Key words : Nigella sativa oral rinse, bleeding on probing, pocket depth

D-2
01-4

Changes in the antegonial angle and depth in the dentate Javanese population

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BACK GROUND : The morphological change in the antegonial region has received little attention in literature. A few studies focused on the antegonial angle and depth of mandible, and there was a relationship between age, dental status, genders and ras. Their result were variable and inconsistent, even using similar methodologies. So far, there was no observation about mandibular antegonial angle and depth in Indonesia especially in Javanese population.

OBJECTIVES : This study analyzed changes in the antegonial angle, antegonial depth in dentate patients in different age groups and between gender.

STUDY DESIGN : A total of 60 patients, who prescribed panoramic radiograph for various purpose were included in the study. The patient were categorized to age and gender. Panoramic radiographs were traced and antegonial angle and depths were measured. Measurements were made by three observers.

ESSENTIAL RESULTS : There were significant differences between right and left side antegonial angle and depth regarding males and females ($p<0,05$). Also no significant differences were observed for the right and left side antegonial angle and depth between 20-29 years and 30-39 years ($p>0,05$).

CONCLUSION : The antegonial angle and depth showed change with gender, that the antegonial angle and depth in males had significantly greater values than females. Furthermore, the antegonial angle and depth did not show change with age. The size of the antegonial angle and depth in Javanese population were within the same ranges of other population.

D-3
01-6

Relationship between dental caries and salivary neutrophil level with nutrition in children

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BACKGROUND : It known that neutrophil is innate imunity. In 4-6 years old children have immature response, so that the intake of nutrients that will adversely affect the sensitivity to various infections such as dental caries.

OBJECTIVE : The objective of this study was to evaluate relationship between dental caries and salivary neutrophil level with nutrition in children.

DESIGN AND EXPERIMENTAL METHODS : Multicenter and observational study were used as study design. Sixty children in kindergarten (4-6 years old) with dental caries and different economic status (low or high) were used as subject sample. Children with free dental caries were used as a control group.

RESULTS : High levels of salivary neutrophil level were associated with dental caries and nutrition ($p<0.0016$ for each comparison). This association remained after controlling some covariables. A salivary neutrophil level decreases in low economic status with dental caries (dmft score>6) and correlated with economic status.

CONCLUSION : From the limited results of this study, it is suggested that in 4-6 years old childern with undeveloped immune response and low number of salivary neutrophil have risk factors for infection as easily dental caries occured and this is associated with nutrient intake as the determiner.

D-4
03-1

Association between periodontitis and rheumatoid arthritis

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BACKGROUND : Periodontitis (PD) and rheumatoid arthritis (RA) are the two diseases which have several similarities. Many studies have shown the correlation of the progression and severity between PD and RA while others reported conflicted results.

OBJECTIVES : To evaluate the inter-relationship between periodontitis and rheumatoid arthritis. Thereby identify the association between the severity of periodontitis and the activity of rheumatoid arthritis.

EXPERIMENTAL METHODS : 100 patients who are diagnosed rheumatoid arthritis from the Rheumatology clinic (RA group) and 100 patients without RA from the General medical clinic (NRA group) as the control group. Both groups are matched in age and gender. The number of tooth loss, plaque index (PlI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) are evaluated in both groups. The activity of RA is evaluated by DAS 28-CRP score and the concentration of anti-CCP antibody.

RESULTS : The incidence of periodontitis in RA group was three-folded greater than NRA group which has significant difference (p=0.000). There was a statistically significant difference in PlI, GI and BOP between two groups. No differences were found in the number of tooth loss, CAL, PPD between RA and NRA. However, the two groups were significantly different in CAL and PPD at levels. The association between the mean anti-CCP antibody and the severity of periodondal disease was also statistically different (P=0.001). In addition, the higher amount of anti-CCP, the higher incidence of severe periodontitis. The difference was significant statistically (P=0.03). There is the relationship between the concentration of serum DAS28-CRP the severity of periodontitis (P=0.028).

CONCLUSION : The inter-relationship is existed between the activity of rheumatoid arthritis and the severity of moderate periodontitis.

D-5
05-2

Compound odontoma in the mandible: A case report

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OBJECTIVES : Odontoma is the most common benign odontogenic tumour of epithelial and mesenchymal origin. Compound odontoma is a malformation in which all of the dental tissues is represented in a pattern that is more orderly than that of the complex type. Enamel, dentine, cementum and pulp are arranged as they would be in the normal tooth. Compound odontoma commonly found in the maxilla approximately 55%.

CASE : A 40 year old female was diagnosed odontoma of the mandible after routine OPG examination. We performed excision of the mass and the post-operative histopathology confirmed the diagnosis.

RESULTS : Operation wound healed unevently. No post-operative complication found. Patient's oral function has no disturbances.

CONCLUSION : Compound odontoma can be found in the mandible. Mass excision still the best option for this condition and shows good result.

Key words : compound odontoma, mandible, excision

D-6
05-4

Ameloblastoma of mandible: a case report

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Ameloblastoma is an odontogenic tumor characterized by aggressive behavior and high reccurence rates with a significant impact on patient morbidity and mortality. The prevalence rate of a large ameloblastoma in our country is approximately 30 patients per year. Our mainstay of treatment ameloblastoma was surgical resection and reconstruction plate placement. In this report we present two cases of large ameloblastomas which manifested as painless swelling. The OPG revealed a large multilocular radiolucency extending from the ramus region to the angulus of mandible with an evidence of destruction in the lower border of mandible. The diagnosis of ameloblastoma was being confirmed by incisional biopsy and the tumor was treated with segmental mandibulectomy and immediate reconstruction with free fibular graft.

Key words : ameloblastoma, mandible, free vascularized fibular graft

D-7
05-5

Management of maxillary ameloblastoma with vascularized bone graft

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Ameloblastoma has been known as a benign locally aggressive tumor. Eigthy percent of ameloblastomas occurs in the mandible while 10,8% occurs in the maxilla. Fifty percents of the case recurred within 1 year of initial treatment. To avoid the recurrences in this case; radical surgery included 1 cm from the margin of tumor also the surrounding bone. This paper is presenting a case report from 49 years old lady with a swelling on upper gum and upper lip, which gave no response to antibiotic treatment. Clinically by extra oral the patient showed facial asymmetry, firm consistency, no palpable lymph nodes, and no tenderness. The histopathologic examination revealed a plexiform ameloblastoma. Maxillary resection with subsequent vascularized bone graft was chosen to manage this case.

Key words : maxillary ameloblastoma, vascularized bone graft

D-8
06-2

Chronic orofacial pain can be extra-territorial: A pain map evaluation

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BACKGROUND : The assessment of pain often utilises multidimensional tools which provide better characterisation of the pain experience. Today, pain drawings have a diverse pattern of usage including in the assessment of localised and widespread pain.

HYPOTHESIS : The extent and distribution of the sites of chronic orofacial pain is paralleled by pain outside the areas of the head and face.

DESIGN & EXPERIMENT : Twenty-eight patients with chronic orofacial pain were selected and interviewed with a set of questionnaires (including anatomical biosketches) via intercept survey. The short form of McGill Pain Questionnaire (SF-MPQ) together with Visual Analogue Scale (VAS) and Behavior Rating Scale (BRS) were used. Patients were asked to indicate painful sites by marking on the anatomical biosketches and also to respond to the SF-MPQ, VAS and BRS. Pain maps were then assessed by the following - Overall pain distribution, spread and laterality, pain sites and pain distribution within the dermatomes (via a clear plastic template). The scores for the SF-MPQ, VAS and BRS were calculated and the relationship with the extent of pain were analysed.

ESSENTIAL RESULTS : Up to 50% of the patients reported pain extending beyond the head and face with 7.4% at the facial aspect, 2% at the frontal and 5% on the back. Beyond the head and face, other areas most affected by pain were the back of the neck, upper and middle back. The pain distribution was asymmetrical with three distinct clusters of dermatomal distributions. There were no statistical significant correlation between the scores of the SF-MPQ, BRS and VAS and the extent of pain.

CONCLUSION : Patients with chronic orofacial pain have more widespread pain than is commonly assumed. Some of these could possibly be comorbid conditions. Pain maps can be a useful tool in the assessment of chronic orofacial pain.

D-9

09-4

Risk factors for excessive overbite and overjet in northeast Thai children

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BACKGROUND : Excessive overjet raises the risk for maxillary incisors injury, while excessive overbite increases bite force between upper and lower anterior teeth leading to severe periodontal disease. Both conditions affect facial appearance and esthetics, which may have an impact on social acceptability and quality of life.

OBJECTIVES : (1) To determine the incidence of excessive overbite (>2/3 of lower anterior teeth length) and overjet (>3.5 mm); and (2) to identify risk factors of these conditions in northeast Thai children.

METHODS : This prospective cohort study included 634 child participants of a birth cohort study conducted in Khon Kaen, Thailand. Mothers were interviewed for risk factor information after delivery, and then every 6 months until the children aged 2.5 years. Examinations for malocclusion were carried out by two calibrated dentists using modified WHO criteria, when the children were 8-9 years of age. Statistical analyses were performed using multiple logistic regression.

RESULTS : The incidence was 12.9% for excessive overbite and 27.9% for excessive overjet. Breastfeeding until at least 6 months of age reduced the risk for excessive overbite by 51 percent (relative risk [RR]=0.49; 95% confidence interval [CI]=0.28-0.87). Children with finger or pacifier sucking habit were twice more likely to have excessive overbite compared to those without (RR=2.18; 95%CI=1.17-4.06). In regard to excessive maxillary overjet, children who were breastfed more than 6 months were 0.28 times as likely to have excessive overjet (RR=0.28; 95% CI=0.17-0.46), compared to those who had less duration of breastfeeding. Finger or pacifier sucking was associated with a twice increased risk (RR=2.18; 95%CI=1.23-3.88), and skeletal class II was associated with a 5.78 times increased risk for excessive overjet (RR=5.78; 95%CI=1.33-25.31).

CONCLUSIONS : Breastfeeding until 6 months of age and avoidance of finger sucking or pacifier use should be encouraged to prevent excessive overbite and overjet in the mixed dentition.

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D-10
09-5

Effects of fluoride and arginine containing tooth paste on dentine hypersensitivity reduction: A randomized clinical trial

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INTRODUCTION : Dentine hypersensitivity is one of symptoms that suffering patients, as a result of several causes, such as gingival recession, abrasion, affraction or erosin of teeth. Fillings, dentine desensitizing agents are used in the treatment of this condition. Currently a lots of tooth paste containing desensitizing agents were introduced to relief this symptom, Arginine is one of the new desensitizing agents however; there is a few clinical study explained the effects of this agent.

OBJECTIVES : The aim of this study was to determine and compare effects of Fluoride and Arginine containing tooth paste on dentine hypersensitivity reduction.

METHODS : The volunteers were introduced to brush their teeth with Fluoride or Arginine containing tooth paste twice a day, visual analog score (VAS score) was used to record hypersensitivity score after applying tactile force and dental unit air stimulation and then estimated personal perception. They were recalled for evaluation VAS score again after using toothpaste 2, 4, 8 and 12 week.

RESULTS : This study revealed that Arginine containing tooth paste can significant reduce sensitivity score at week 4, 8 and 12 when use tactile stimuli and volunteer's personal perception compare with baseline visit ($p<0.05$), for dental unit air stimuli Arginine containing tooth paste can reduce sensitivity score at week 8 and 12 compare with baseline visit ($p<0.05$). Arginine containing tooth paste can reduce sensitivity score more than fluoride containing tooth paste at week 4, 8 and 12 when use tactile stimuli and dental unit air stimuli, at week 12 when use volunteer's personal perception ($p<0.05$).

CONCLUSION : Collectively, our data show that Arginine containing tooth paste can reduce sensitivity score more than fluoride containing tooth paste.

D-11
10-1

Implant supported and implant-tooth supported overdenture (Hybrid telescopic double crown concept)—Case report

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Implant overdentures are economical and useful to fabricate osseointegrated prostheses that provide a significant improvement in stability, retention, bite force, and chewing efficiency compared to conventional dentures.

The appropriate choice of attachment can be made on the basis of the given anatomical state of the mandible and maxilla. In maxillary implant prosthodontics, bar attachments are the standard retaining devices for removable dentures. Their rates of success are high, but the splinting of the implants with the bar requires meticulous oral hygiene and may pose problems if an implant fails

In mandibular prosthodontics, advanced atrophy of the alveolar crest calls for prosthesis stabilization especially with regard to horizontal forces; this is best achieved using bars or parallel-walled telescopic crowns.

Double crowns are used frequently to retain removable partial dentures. In comparison to other retention devices (eg, clasps, bar or ball attachments), the advantages of double crowns include superior esthetics, good retention, better stability, and expansion possibilities.

According to the retention mechanism, double crown can be subdivided into telescopic crowns, conical crowns and double crown with a clearance fit. The double crown with a clearance fit was also called hybird telescope or hybrid double crown. Telescopic crowns use the friction of the surfaces of the inner and outer crowns, conical crowns use the wedging effect for retention, and double crown with a clearance fit use the additional attachments or functional molded denture borders for retention. We use hybrid telescopic double crown with friction pin.

Recently implant and tooth overdenture in which implants were placed strategically to improve stability revealed high success rate. I want to report to hybrid telescopic implant supported overdenture and implant-tooth supported overdenture.

D-12
11-1

Seropositivity of HPV 16 E6 and E7 and the risk of oral cancer

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BACKGROUND : Oral squamous cell carcinomas (OSCC) is a serious and growing health problem in many developing countries representing high mortality rates of up to 50%. Smoking, alcohol consumption and betel quid chewing habit are the well established risk factors of OSCC. However, there is a number of OSCC patients without any risk habits suggesting the presence of other causative factors such as Human Papillomavirus (HPV) infection. Although getting more evidences indicate the involvement of HPV infection and OSCC, but the etiological role of HPV in OSCC still remain controversy.

OBJECTIVES : To determine the prevalence of HPV 16 seropositivity among OSCC patients and healthy normal individuals using a glutathione-s-transferance (GST) capture HPV 16 ELISA and hence examine the association between HPV 16 seropositivity and risk of OSCC.

MATERIALS AND METHODS : HPV 16 E6 and E7 plasmids were constructed for the production of recombinant protein which was used as the antigen in the ELISA assay. Then, GST capture HPV 16 ELISA was optimized and serum samples from 50 healthy individuals and 50 OSCC patients were tested using this ELISA assay. Multiple logistic regression tests were used to examine the association between HPV 16 seropositivity and risk of OSCC.

RESULTS : Using the HPV ELISA, 30% (OR=2.25, 95% CI=0.85-5.93) and 18% (OR=1.61, 95% CI=0.53-4.92) of oral cancer patients were found to be HPV 16 E6 and E7 seropositive respectively. Significant association was found between HPV 16 seropositivity and elevated risk of OSCC in men but not in women subjects (OR=21.74, 95% CI=1.30-333.33). A similar trend was observed in non-betel quid chewers.

CONCLUSIONS : The potential associations between HPV 16 E6/E7 seropositivity and oral cancer were revealed in men and non-betel quid chewers subjects suggesting a possible etiological role of HPV 16 in subgroup of OSCC patients in Malaysia.

D-13
12-1

Clinico-pathological presentation of oral leukoplakia in Sri Lankan patients: Analysis of 742 cases with diagnostic biopsies.

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BACKGROUND : Leukoplakia is a clinical entity which could be histopathologically diagnosed as a lesion with or without epithelial dysplasia. Betel quid chewing/ smoking are the commonest cause of leukoplakia in Sri Lanka.

OBJECTIVES : Objectives of this retrospective study were to analyze the clinical presentation of leukoplakia including age, sex, and site of the lesion and correlate the findings with that of histopathological diagnosis of respective lesions.

MATERIALS AND METHODS : A total of 742 biopsies received by the Department of Oral Pathology, Faculty of Dental Sciences/University of Peradeniya with a clinical diagnosis of leukoplakia erythroleukoplakia, speckled leukoplakia, precancerous lesions, and premalignant/ dysplastic lesions were selected for the study. Out of the total, 656 white lesions with a histopathological diagnosis of keratosis with or without out dysplasia were analyzed separately.

RESULTS : Of the total 86 (11.6%) were histopathologically diagnosed as squamous cell carcinomas and were excluded from further analysis. Majority of the specimens were from males (77.9%) with a male to female ratio of 3.5:1. Buccal mucosa was the most common site of occurrence (72.3%), followed by the tongue (13.3%). Leukoplakias in elderly patients were found to contain significantly higher grades of dysplastic changes compared to younger patients (χ^2= 53.2, P=0.001). Males were more likely to present with dysplastic lesions than females (χ^2= 8.2P=0.04). Further, lesions clinically diagnosed as speckled leukoplakia and erythroleukoplakia presented with a higher degree of dysplasia, compared to homogenous leukoplakia (χ^2= 30, P=0.001).

CONCLUSION : Present study reveals that a higher degree of dysplasia or malignancy is likely in elderly patients and or with lesions clinically diagnosed as speckled leukoplakia/ erythroleukoplakia. Such patients require early surgical intervention as the best management strategy. Therefore alerting clinicians on present findings would be useful to select the best management strategy for their patients.

D-14

15-3

Stress analysis of mandible ameloblastoma by 3-dimensional precise reconstruction model and rapid prototyping solid model

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BACKGROUND : Ameloblastoma is a benign tumor, commonly happened in jaw-bone, and about 1% of oral cancer disease. Although it showed benign on histology, its high recurrent rate was caused by inadequate resect. Oral is a complex mechanical environment and local mechanical effects may play an important role in stimulating tumor cell spreading.

OBJECTIVES : The objective of this present study is using finite element analysis (FEA) to investigate the principal stress and stress distribution of a 3D precise reconstruction model and a rapid prototyping (RP) solid model of an ameloblastoma patient, providing information that would be valuable in dental and biomedical applications.

EXPERIMENTAL METHODS : Stress analysis models were reconstructed from volume computed tomography data (VCT). The interval of which is only 0.625 mm were reconstructed model could be very precise and we could differentiate the scope of tumor. Using FEA program (ANSYS Workbench 12.1) to mesh 3D model and stimulate the occlusion condition as boundary conditions. Moreover, convert 3D model into STL (stereolithography) file to build a solid model with RP machine (ZPrinter 450, Z Corp.) to analyze the mechanical tests was performed.

RESULTS : The ameloblastoma spreading in the middle of mandible left lateral incisor and canine caused the frontal teeth malalignment, so the maximum stress was concentrated on these two teeth middle. However, the ameloblastoma made the cortical bone surrounding the tumor thinner to cause more stress on bone during occlusion. The results of principal stress show significant tension stresses on the tumor that brought mechanotransduction with oncogenic signaling pathway in tumor cell spreading.

CONCLUSION : Our study shows that this could be potentially benefits for understanding the stress properties of mandible ameloblastoma during occlusion. Otherwise, the RP solid model could be a preoperative model to help the surgeon plan and shorten the operative time (and therefore shorten the wound exposure time and decreased blood loss).

D-15
15-5

Microstructure characteristics and biocompatibility of laser surface-modified austenitic stainless steels containing micro/nano-porous layer for biomedical applications

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BACKGROUND : The types of 316 and 316L austenitic stainless steels are widely used in industrial and medical devices such as mini-implants, bone screws, bone plates, orthodontic brackets and acupuncture needles etc. because of their excellent strength and stiffness, corrosion and oxidation resistance, and superior workability and good biocompatibility. Recently, a non-contact laser processing was applied in surface modification of implant materials in order to generate some functional surface properties, without sacrificing any useful mechanical or biological properties.

OBJECTIVES : The purpose of the present study is to investigate the influence of laser rate on the microstructure and biocompatibility of the laser-modified (LM) SUS 316 stainless steels for biomedical applications.

EXPERIMENTS METHORDS : The SUS 316 stainless steel substrates were LM at 300 W for different speed rates. The superficial properties and microstructure of the LM samples were investigated by means of optical microscope, scanning electron microscope, atomic force microscope transmission electron microscope, and contact angle goniometer. Moreover, cell cytotoxicity assay of LM samples was evaluated as per ISO 10993-5 specifications.

RESULTS : After treated with various laser speeds, the recast layer with micro/nano porous structure formed on the surface of specimens. Moreover, morphologies on the recast layer changed from hole-like structure to wave-like structure as the laser treatment speed increased. Thicknesses of the recast layer of the specimens are approximately 0.5 to 1 μm. Cell cytotoxicity test also demonstrated that the LM samples possessed the excellent biocompatibility.

CONCLUSION : After laser treatment, the surface roughness of samples significantly increased. Morphologies on the recast layer changed from hole-like structure to wave-like structure as the laser treatment speed increased. Moreover, the MTT assay results exhibited that the LM samples possessed good biocompatibility. The authors would like to thank the Southern Taiwan Science Park Administration and Biomate Medical Devices Technology Co., Ltd. for financially supporting this research under contract No. EI-33-09-24-101.

D-16

15-6

Semi-tubular implant surgical guide system for dental implant in posterior regions with leaderguide system

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BACKGROUND : The present study relates to a new surgical guide for dental implant which is different from the conventional ones. The semi-tubular design makes it easier to approach during surgery and let the dentist to directly look at the drill to identify the depth of drilling.

OBJECTIVES : This study is to investigate the accuracy of positioning the implantation in the posterior regions of mouth through the application of semi-tubular surgical guide system - Leaderguide implant surgical guide system.

EXPERIMENTAL METHODS : 30 patients need posterior implant restorations who were taken by impression and the study models were calculated for the available spaces, and survey on surveyor to determine the positions of implants with references of previous X-ray, periapical or panoramic, then positions check guide and surgical guide were constructed with Leaderguide and checked X-ray. After implant surgery, the implant positions were checked with periapical or panoramic X-ray. The accuracy of the implant positions were analyzed from M-D direction of the X-ray.

RESULTS : The accuracy analyzed from X-ray showed 96% implants were placed in ideal positions.

CONCLUSION : Semi-tubular implant surgical guide, Leaderguide, is an accurate surgical guide for dental implantation through the evaluation of periapical or panoramic X-ray examination in clinical research. This study was analyzed with 2D X-ray only, further analysis with 3D computerized tomography may be necessary.

D-17

16-1

A multi-center survey: oral healthy behavior and risk factors of dentine hypersensitivity

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BACKGROUND AND RATIONALE : Most of the previous studies on oral healthy behavior and risk factors of dentine hypersensitivity have been carried out in western countries, while only limited data on samples of Asian populations are available.

STUDY OBJECTIVE : The objective of the present study was therefore to carry out a cross-sectional and multi-center survey on oral healthy behavior and risk factors of dentine hypersensitivity in the Chinese population.

STUDY METHODS : The national survey was a multi-centre and random sampling investigation in urban districts and rural areas of eight provinces in China: Beijing, Shanghai, Chongqing, Sichuan, Hubei, Shanxi, Guangdong and Liaoning. Adult subjects aged 20-69 years old were investigated and divided into five age groups. All subjects were examined by one practitioner in each city. Questions about oral healthy behavior and dentine hypersensitivity were read to the subjects, and the answers were recorded by an assistant. All subjects were clinically examined for dentine hypersensitivity by evaporative (air) sensitivity assessment.

RESULTS : The study presented that the population in urban districts showed better oral health behavior than those in rural areas (including the frequencies of collecting information of oral health, buying products of protecting oral health and visit the dentist regularly, $P<0.01$). The population with higher social-economic background paid more attention into maintenance of oral health than those with less social-economic background. Binary logistic regression presented that the single variables associated with the occurrence of dentine hypersensitivity include the following: gender, frequencies of toothbrushing, duration of a toothbrush used, reason of exchanging a toothbrush, type of toothbrush's stiffness, gastroesophageal reflux disease, frequencies of having white sprint, and frequencies of having fruit juice.

CONCLUSION : Oral health behaviors in different population were found to vary and risk factors of dentine hypersensitivity in Chinese population were also diversified.

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17-1

Effect of connective tissue graft (CTG) on gingival recession before palatal orthodontic movement of buccally erupted canine

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BACK GROUND : Labial orthodontic movement has been considered a risk factor of gingival recession. When tooth moves labially, facial gingiva is usually getting thinner. Thin labial plate and gingival tissue are more prone to gingival recession. Therefore, labial orthodontic movement, CTG has been performed to prevent thinning of gingival tissue. And, when tooth moves back palatally, facial gingiva and alveolar bone are getting thicker. The thicker gingiva is, the more resistant to inflammation and gingival recession. However, in clinic, gingival recession has been found during palatal movement of buccally erupted canines with thin gingiva.

OBJECTIVES : The purpose of this study was to evaluate effect of connective tissue graft (CTG) before orthodontic treatment of buccally erupted maxillary canine.

EXPERIMENTAL METHODS : 20 cases with buccally erupted maxillary canines were selected. In 10 cases (grafted group), CTG was performed on canines before orthodontic treatment. Mean 3.7 months after CTG, orthodontic wires were applied. While the other 10 cases (non-grafted group) received no graft before orthodontic treatment. Gingival recession was calculated by measuring clinical crown length on diagnositic cast before and after orthodontic treatment.

RESULTS : At base line, clinical crown length showed no statistically significant difference between grafted group and nongrafted group ($P>0.05$). However, the crown length changes during orthodontic treatment were significant between two groups ($P<0.05$). In grafted group, gingival margin moved coronally (mean 0.55mm) after orthodontic treatment, but not statistically significant ($P>0.05$). While in non-grafted group, gingival margin shifted apically (mean 0.57mm), and it is statistically significant ($P<0.05$).

CONCLUSION : The use of CTG is predictable to prevent gingival recession after palatal orthodontic movement of buccally erupted maxillary canine.

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18-4

Oral health education for children in rural areas of Cambodia

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BACKGROUND : In Cambodia, especially in rural areas, many children do not have opportunities to learn about oral health in spite of having extensive caries. We visited a primary school in Siem Reap Province 6 times to offer instructions and guidance on oral health and hygiene. But one school we visited has about 5,000 students; therefore, we could not teach them individually during our visit.

OBJECTIVE : Our objectives were to help improve the state of oral hygiene and establish a prevention program, thus, realizing nationwide independent dental care.

DESIGN : First, we investigated the oral health conditions of Cambodian children and treated cases of caries. Then, we provided oral health education in cooperation with a local school. We instructed the teachers, who will then be able to teach the children without our support. We demonstrated our trial class procedures to the teachers by using the Khmer language through an interpreter. We educated them about the importance of maintaining their teeth to keep them healthy. Next, we told them how to prevent oral cavities from dental diseases by rinsing their mouth or brushing their teeth. We made sure every student understood our slogans concerning sugar control and brushing.

RESULTS : As a result, they established a brushing routine after meals and repeatedly instructed their students on the slogans of sugar control and brushing. Now, everyone in that primary school is interested in maintaining good oral health.

CONCLUSIONS : Now, we plan to educate trainee teachers in a teacher training school in Cambodia, so that they will be able to teach oral health to their students. And we also conducted a questionnaire survey for better understanding the diet and lifestyle, thereby aiding in developing different ways for offering oral health guidance in future. We think that these activities will lead dentistry in Cambodia to become independent.

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18-24

Oral care support for children with type 1 diabetes mellitus

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BACKGROUND : Type 1 diabetes mellitus (DM) is considered to be a risk factor not only in general medical but also in oral diseases. We continue to support children with type 1 DM who attend summer camp to practice self-management for their disease through group living with children with the same disease. Our dental support in the summer camp started at 2005. We check the children's oral condition and provide individual guidance on oral care.

In this study, we compared children's oral conditions between 2011 and 2005 to consider ways to improve our support.

OBJECTIVES : The subjects were 20 children with type 1 DM (7-14 years old) who attended summer camp in 2011 (2011DM), 28 children with type 1 DM (7-14 years old) who attended summer camp in 2005 (2005DM) and 168 children (7-14 years old) who visited Hiroshima University Hospital Pediatric Dental Clinic (non-DM). We also used the national average from a report on the survey of dental diseases (2005) (JPN).

EXPERIMENTAL METHODS : 1 Periodontal condition: BOP and CPI were assessed on 61┘, └6, 6┐, ┌16 as teeth being tested.

2 Plaque sampling, genomic DNA and PCR for bacterial species caused periodontal disease: dental plaque was collected with a sterile toothbrush for 1 minute from erupted teeth. Genomic DNA from each plaque sample was obtained using a standard miniprep procedure. PCR was performed using species-specific primers, as described previously. *Tannerella forsythia* (*T.f.*), *Treponema denticola* (*T.d.*), *Prevotella intermedia* (*P.i.*), *Porphyromonas gingivalis* (*P.g.*) and *Aggregatibacter actinomycetemcomitans* (*A.a.*) were detected with electrophoresis after PCR.

3 Correlations between BOP and HbA1c (JDS values) were investigated and the X^2 test was used to examine detection rates of bacterial species in DM and non-DM groups.

RESULTS : 1 Higher rates of CPI≥1 were found in both DM groups (2011DM: 80.0%, 2005DM: 96.4%, JPN: 46.3%). 2011DM showed more favorable results than 2005DM.

2 Comparing the prevalence of BOP+ between 2011DM and 2005DM, there was a significant decrease in 2011DM ($p<0.01$). Concerning the number of BOP+, there was also a significant decrease in 2011DM ($p<0.01$). However, there was a tendency whereby the number of BOP+ increased in association with higher values of HbA1c in both DM groups.

3 There was no difference between 2011DM and 2005DM regarding detection rates of bacteria species.

CONCLUSION : From the comparison between 2011DM and 2005DM, it was suggested that the oral condition of children with type 1 DM was slightly better due to our continued educational activities to promote oral health.

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18-25

Dental support activities collaborated with local systems in rural area of Cambodia

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BACKGROUND : In Cambodia, especially in rural areas, many children have extensive caries, but they do not have opportunities to receive dental treatment because of a shortage of dentists, poverty, and lack of dental knowledge.

OBJECTIVE : To improve Cambodians' oral health, we visited Cambodia six times since 2009 and performed dental support activities at several primary schools.

Our goals are to shift from the "treatment" to the "prevention" and to supply dentistry by Cambodian people themselves.

DESIGN AND ESSENTIAL RESULTS : At first, we examined their oral health conditions and treated them for simple dental caries. There are many cases of dental caries, and most of them are untreated. Furthermore, the decayed, filled primary teeth (dft) score is also high. For example, the mean dft score in 7 years old students is 8.4. So, the current need in Cambodia is for "treatment." However, "Oral Health Education" is necessary as a basic solution.

Therefore, we provided oral health education in cooperation with a local school and hospital. We also have started collaborating with dental students of the University of Health Sciences of Cambodia and the Ministry of Health of Cambodia. We have performed our activities with them and have interacted with them about oral health education.

On the other hand, about 50 students from Hiroshima University Faculty of Dentistry participated in these activities by now. It may be said these experience, to see and feel the real needs of dentistry, has been something very precious and been impressed to them.

CONCLUSION : Our efforts should lead to an awareness of oral health needs among more people. We think that these activities will lead dentistry in Cambodia to become independent. We hope Cambodians gradually begin to show self-improvement in their oral health conditions.

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18-32

The Inhibitory effects of bovine lactoferrin on growth and invasion of oral squamous cell carcinoma

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INTRODUCTION : Lactoferrin (LF), an iron binding milk protein, has been reported as anti-tumor, anti-inflammatory, anti-bacterial, anti-viral, and immunoregulatory effects. Although some studies have shown inhibitory effects of LF on tumor growth and tumor malignancy of various cancer types, its mechanism remains to be clarified.

MATERIALS AND METHODS : Some non EMT and EMT induced cell lines, HOC313 and HSC3, were used to investigate the inhibitory effects of bLF on OSCC. Its effects on tumor cells growth, cells invasion, and cells migration, were assessed, with doses dependent manner of bLF of 1 μg/ml, 10 μg/ml, and 100 μg/ml, using proliferation analysis, invasion assay method, scratch migration assay, respectively. The mechanisms of bLF on cells invasion were explored using specific pathway inhibitor. The involvement of lactoferrin receptor low-density lipoprotein receptor-related protein 1 (LRP1) was examined with RNA interference technique.

RESULTS : We reveal the some new insights of the inhibitory effects of LF on oral squamous cell carcinoma (OSCC) and related to subcellular mechanism. Our findings showed that of bLF suppressed cells proliferation to all examined OSCC cell lines and induced apoptosis in a dose dependent manner. More importantly, with bLF treatment, E-Cadherin significantly increased in both mRNA and protein levels of HOC313 which then lead an inhibition of cells migration and cells invasion. In LRP-1 knockdown cells, bLF neither exert inhibitory effects on OSCC cells invasion nor regulate E-Cadherin expression.

CONCLUSION : Together, our data suggest that bLF may regulate LRP-1 mediated signal transduction, resulting in the suppression of OSCC cell proliferation, migration and invasion. Considering the obvious effects of LF on tumor, LF could be used as an anti-cancer therapeutic agent.

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18-33

Bovine lactoferrin enhances osteogenesis through TGF-β receptor signaling

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OBJECTIVES : The pleiotropic functions of bovine lactoferrin (bLF) are known but poorly understood. bLF has been reported to stimulate osteoblast proliferation, enhance thymidine incorporation into osteocytes, and reduce apoptosis of osteoblasts. However, the essential effects of bLF on bone cell anabolism and related mechanism are not well demonstrated.

METHODS : C3H10T1/2, a mouse mesenchymal cell line, and primary osteoblasts were cultured in α-MEM. *In vitro* study, alkaline phosphatase (ALP) activity, mineralized nodule formation as well as the expression of osteoblast differentiation markers were examined. Western blotting analysis, immunoprecipitation and binding assay were performed to clarify the bLF-induced signal transduction mechanisms. *Ex vivo* organ cultures of mouse calvaria were also performed.

RESULTS : bLF enhanced ALP activity and the expression of early osteoblastic differentiation markers, *Runx2*, *ALP* and *Osterix*, in C3H10T1/2. bLF also up-regulated ALP activity, mineralized nodule formation and the expression of late osteoblastic differentiation markers, *BSP* and *OCN*, in primary osteoblasts. Furthermore, bLF induced Smad-dependent and Smad-independent MAPK activation. Both ALP activity and mineralized nodule formation induced by bLF treatment were eliminated in TGF-β receptor (TβR)-I or p38 kinase inhibitor treated cells. The direct binding of bLF to TβR-II was also observed. In *ex vivo* experiments, it was revealed that bLF promoted new bone formation and regenerating of bone defects.

CONCLUSION : Our data suggest that bLF is a potent osteogenic factor, which exerts its actions by activating the TGF-β signaling pathway. Our data indicate that bLF has distinct anabolic effects on the development and growth of osseous tissue in mammals. We anticipate that bLF will be a valuable agent for bone regeneration.

AUTHOR-INDEX

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※University emblems are placed by order of international exchange agreement (both university and faculty/school levels) concluded from the oldest to the most recent. Those of institutions having no agreement on academic exchange with HU are put at the end in alphabetical order.