Exploring the Determinants of Osseointegration: Interview with Drs Ichiro Nishimura and Takahiro Ogawa

by Dr John Beumer

Osseointegration has had a profound effect on our profession. Until recently, the biologic foundation upon which this phenomenon was based relied mainly on light microscopic studies. However, in the past several years, Ichiro Nishimura and Takahiro Ogawa of UCLA's Weintraub Center for Reconstructive Biotechnology sought to explore the biochemical and genetic events that control the osseointegration process. Drs Nishimura and Ogawa are both qualified prosthodontists with PhDs and an expertise in molecular biology. Dr Nishimura joined the UCLA faculty in 1997 and soon after established the Weintraub Laboratory. Dr Ogawa's postdoctoral student status in 1998 eventually led to a full-time faculty appointment at UCLA. Their studies showed that titanium surfaces with specific surface topographies accelerate and/or upregulate genes that are associated with bone repair and differentially induce the expression of genes that are unique to the process of osseointegration (the so-called osseointegration genes: TO-1, TO-2, TO-3). Their research has provided valuable insights that explain why these specific surface topographies accelerate the process of osseointegration and why the bone deposited is harder and stiffer than bone deposited on machined-surface implants. The following interview with Drs Nishimura and Ogawa was prepared by Dr John Beumer, who is head of prosthodontics at UCLA and a long-standing member of *IJP*'s editorial family. The interview seeks to provide the reader with a sense of our colleagues' initial forays into science and their subsequent breakthrough scientific studies with respect to osseointegration.

(left to right) Drs Takahiro Ogawa, Ichiro Nishimura, and John Beumer.

How did each of you come to be interested in a career in dentistry?

Nishimura: My father, Dr Shichiro Nishimura, is a graduate of the Tokyo Dental College and influenced me to attend his dental school. He was the seventh of 8 siblings from a family with a humble background in downtown Tokyo. I believe he is one of the few of our family members to obtain higher education. After he started his solo practice, he became involved in microbiology research at the Toho Medical University and earned a PhD as a part-time graduate student. He then spent a year at the University of Southern California as preceptor in prosthodontics in the 1960s. He brought back not only the lost wax casting method and the neutral zone denture concept, but also a great enthusiasm for the US postgraduate training system.

Ogawa: I was an engineering-oriented person. I loved to assemble plastic models, and always dreamt that "some day I will invent the world's fastest car." One day during my late high school days, I got a revelation that it was my mission to talk to people and work for people. To me, dentistry seemed to be the best blend of making things and interacting with people, and so I applied to Kyushu University School of Dentistry in 1984. After over 20 years, now I feel that my old interests are reviving and guiding me back in the engineering direction, which is nowadays called biomaterial sciences, such as the development of new implant surfaces and bone substitute materials. Ironically enough, current car technologies feature machines interacting with humans, such as driver-responsive transmission control and voice-activated navigation systems. I hope I will not regret my decision and say, "I should have worked for Toyota."

Dr Nishimura, what factors encouraged you to pursue a career in dental research?

Nishimura: After my graduation from Tokyo Dental College in 1981, I joined the Department of Prosthodontics as an instructor. This was roughly equivalent to a US residency program. My mentor, the late Prof Hiromu Sekine, was one of the pioneers of Japanese prosthodontics, and he encouraged me to consider an academic career. He had been impressed with Prof Douglas Atwood's lecture at the International Assocation for Dental Research meeting in Osaka, Japan, and suggested that I go to Harvard and study pathophysiological mechanisms of residual ridge resorption.

Please provide some comments on the influence Doug Atwood had on your career.

Nishimura: Upon my arrival at Harvard in 1982, Prof Atwood invited me and my co-resident to his tutorial research seminar and shared his expertise on ridge resorption. This intense and personal tutorial provided a most catalytic influence on my career. I learned not only the subject matter, but more importantly, the commitment required of a mentor to his students. After the 1-year tutorial, we were obliged to submit a term paper in the form of a research proposal, which later became the backbone of my doctoral dissertation research. Currently, I offer small group seminars to the UCLA college freshmen as part of general science education, including "Science of the FACE" and "Research Management for Young Scientists and Creative Minds," which are designed following the traditional yet conductive style of Prof Atwood's tutorial.

Prof Bjorn Olsen at Harvard also played an important role in your development as a scientist. How did you become involved in his research lab?

Nishimura: The academic environment at Harvard's Prosthodontics Department encouraged us to cross many

different academic disciplines. For example, I took a didactic class on skeletal evolution offered by the Comparative Zoology Department. In the class, Prof Bjorn Olsen, a young maverick scientist from the Department of Anatomy and Cellular Biology of Harvard Medical School, showed how molecular cloning of new collagen genes critically advanced the fundamental understanding of cartilage development. I became a frequent visitor to Prof Olsen's laboratory, which turned out to be next door to the dental school.

One day near my graduation, Prof Olsen asked me if I would like to join his laboratory as a research associate. Becoming a full-time scientist had never crossed my mind before, and my fellow dental school graduates were busy interviewing for clinical positions. Therefore, it was a surprise to me that all of my mentors strongly encouraged me to take this opportunity. I called my father in Japan for his guidance. Perhaps from his previous aspirations in science, he also supported this advanced research training. This was when I decided to take Prof Olsen's offer of an NIH stipend as a full-time research associate in the field of molecular biology. Retrospectively, this was the most exciting, satisfying, and fulfilling time of my life. I believe that my father intended to have his son come back to his practice in Tokyo; however, my parents continue to support my career decisions.

Dr Ogawa, how did you come to be interested in dental research?

Ogawa: During my dental school education, I enjoyed treatment procedures and concepts associated with prosthodontics, including occlusion, mandibular function, and osseointegrated implants. However, research, which I understood was to explore and discover biological phenomena and treatment modalities, was also very attractive to me. I was sick of following the textbook scenarios of dentistry. After debating for several months, I decided to pursue a PhD focusing on mandibular function and occlusion at Kyushu University. Despite some successful publications in that field, I started to feel uneasy about the direction of my research, because my research did not seem to contribute to improving tomorrow's dental treatment. Since then, I cultivated my ideas based on problem-oriented research (a problem-solving approach rather than a method-based approach) and multidisciplinary research, which led me to incubate a concept of "biology-driven prosthodontics." It was my red letter day on March 15, 1997 in San Francisco when I happened to meet Dr Ichiro Nishimura, a prosthodontist with expertise in biology.

What impact have your parents had on your approach to your career?

Ogawa: My parents always supported my decision to pursue an academic career. A pivotal event that impacted my life was when my parents sent me to learn Kendo. I was 5 years old at the time and continued to play until my graduation from dental school. Kendo is the Japanese version of fencing or swordsmanship. After long and hard practice, I won a championship in Nagasaki prefecture and took third place in the nation when in high school. Kendo requires sportsmanship, fair play, and a must-win frame of mind. Unfortunately, working in academic and science worlds, there was no such notion as opponents or combat. What I learned from Kendo, however, was to maintain my passion and constantly attack temptations to laziness and compromise. This is where I return every time I get lost.

Describe the importance of your wife and daughter in your decision to stay in the United States and pursue an academic career.

Ogawa: I came with my wife and daughter to Los Angeles in the summer of 1998. We planned to return to Japan in 2 years. However, in 2000, while I was in the middle of gene analysis on osseointegration, our family was confronted with a crucial choice that would decisively affect our lives. If I stayed longer in the US, I had to quit an assistant professor position in Japan. We debated for a full 3 months. The greatest and most thankful thing to me was that my wife and daughter supported my decision to remain in this country. There is no doubt that living in a foreign country with a different language and culture requires a tremendous effort to adapt. My wife had developed a very successful career in Japan as a pharmacist. But once we remained in this country, she had to virtually abandon her profession, and she was not entitled to work in the US because of our visa status. During my years as a postdoc and research associate, there was no guarantee I would obtain a stable position in the future. We just took a chance. I was already 36 years old at the time, which is supposed to be an age when a man has established a good financial status. However, my wife did not complain at all and continued to encourage me.

Almost from the beginning of your careers, both of you expressed an interest in the process of osseointegration. Please describe your early studies and how they led you to embark on the effort to uncover the secrets of osseointegration?

Nishimura: My first encounter with osseointegration was in Japan in 1981 soon after I joined the faculty at the Tokyo Dental College. One of our senior faculty, Dr Yataro Komiyama, was then studying in Sweden under Prof Per-Ingvar Brånemark. We had become aware of osseointegration-based implants through Dr Komiyama. From 1982 to 1986, I had an opportunity to study prosthetic dentistry at the Harvard School of Dental Medicine, where an NIH-funded clinical trial on implant treatment outcomes was ongoing. In this study, the effectiveness and survival rate of blade implant–supported mandibular posterior fixed partial dentures was being investigated. This study was a response to the Harvard-NIH consensus meeting on dental implants, which assessed various implant systems including blade implants and subperiosteal implants. Clinical studies on implant systems were just beginning in the US at the time, but the evolving information was still sketchy and often lacked objective evaluation.

When I started my own laboratory at Harvard, one of my research goals was to elucidate the molecular mechanism of remodeling and resorption of alveolar bone of edentulous jaws. The first task was to establish reliable animal models useful for molecular biological research.[1,2] Using rodent models, we developed an in situ hybridization protocol of labeled DNA probes suitable for alveolar bone histologic sections in order to determine the cellular source of certain gene products. Osteoblasts in alveolar bone appeared to bypass the formation of precursor cartilage callus during tooth extraction wound healing and directly deposit bone matrix such as type I collagen and osteocalcin.[3] However, further studies revealed a puzzling fact, namely, that alveolar bone osteoblasts did express cartilage genes. We later found that one such cartilage gene product, col9a1 (alpha 1 chain of type IX collagen), is truncated due to alternative promoter activation in osteoblasts, which we believe explains why the

tooth extraction socket is not filled with cartilage,[4] as often seen in long bone fracture healing. These findings provided insights into the complexity of alveolar bone remodeling and suggested that placement of dental implants into this complex biological system could significantly influence and perhaps alter cellular behavior.

Ogawa: I was very fortunate to join Dr Nishimura's research team in 1998. After 3 years of training as a postdoctoral researcher, I started my own laboratory (Laboratory for Bone and Implant Sciences, a subsection of the Weintraub Lab) in 2002 and joined the faculty at UCLA. During the transitional period from postdoctoral training to independent researcher, I decided to pursue the concept of biology-driven prosthodontics in my research. I strongly felt the necessity to apply scientific biological methodologies to prosthodontics research and implant research in particular. When I began my implant research, manufacturers were eager to develop and commercially promote dental implants that were more bioreactive and to support researchers who generate data to support their claims. In my mind, one of the principal roles of my research team was to validate the existing knowledge and provide answers to issues under debate. Fortunately, we successfully unveiled many aspects of osseointegration by simply asking ourselves why such a phenomenon occurred.

Our group was governed by the principle of "elementalism." Elementalism, a principle in physics, is an attempt to explore truth by reducing phenomena to their basic elements. We therefore felt that the data obtained at molecular and atomic levels should be more revealing. We decided to explore issues not yet addressed in the science of osseointegration by taking advantages of molecular genetic biology technologies that had rarely been used in the field up to that time. These new approaches helped us to uncover the secrets of osseointegration and moved the science to a higher level.

One of your original thoughts was that gene expression is controlled at local levels by the surface texture of the implant. What led you to develop this hypothesis?

Nishimura: It is well documented that implants with roughened surfaces, when integrated with bone, resist stronger dislodging forces compared to the machined-surface implants, at least in the short-term and in laboratory animals. This was felt to be due to the bone tissue penetrating the niches of roughened implant surfaces, thereby creating a better "grip." However, we were fully aware that cells can sensitively respond to the substrate topography through so-called contact guidance. While creation of the osteotomy should induce bone wound healing in general, we postulated that cells in physical contact with implant surfaces may modify their behaviors through contact guidance. Therefore, the surface topography must be an important modifier of cell behavior and highly relevant to the establishment of osseointegration.

Ogawa: One of our concerns regarding the previous implant research was the lack of evidence with regard to the "speed" of osseointegration. Although many manufactures' brochures and related publications claimed faster osseointegration of their particular implant system, little data had been provided to support these claims. Suppose that more bone was found on implant surface A than on implant surface B at 1 month of healing. Does this mean that implant surface A induced bone formation faster than implant surface B? Fragmentary observations at the time using histology or other morphologic methods did not provide sufficient evidence to reveal the "speed" of osseointegration. Our idea was, "Why not ask the cell?" Osteoblasts express specific genes, ie, messenger RNA, in line with their level of functional maturation. For instance, the expression of type I collagen starts at the early differentiation stage, while the expression of osteocalcin is initiated at a later stage. Therefore, the analysis of the expression of bone-related genes can tell us how fast osteoblasts are maturing and how fast the bone formation is proceeding.

Based upon the well-established phenomenon that the percentage of bone-implant contact is higher for the acid-etched, microroughened surface than for the machined surface,[5] we were motivated to conduct genetic analysis studies of the bone around implants with these 2 different surface textures. We demonstrated that the expression of bone-related genes were accelerated and/or enhanced around the acid-etched surface compared to the machined surface, for the first time providing evidence to support the hypothesis that bone formation around the acid-etched surface is accelerated.[5–7]

What led you to hypothesize that a set of genes not involved in bone repair or regeneration initiate and/or regulate the process of osseointegration?

Nishimura: Our initial experiments were quite interesting. We were attempting to develop an intraoral implant rat model. We fabricated small cylindrical titanium implants 1 mm in diameter and 2 mm in length, and surgically placed them in the rat maxilla anterior to the first molar (generally there is an edentulous space in this area). Rats have 3 molars on each side of the maxilla. Unlike the continuously growing incisors, rat molars are supported by well-developed periodontal tissue similar to humans. The maxillary first molar has a large mesial root extending anteriorly. In our study, several experimental implants contacted the mesial root of the maxillary first molar, and were surrounded by a soft tissue resembling the periodontal ligament. The functional periodontal ligament contains a specific set of extracellular matrix molecules similar to those found in ligaments, and type XII collagen is one such periodontal ligament–associated matrix molecule.[8] We examined the expression of the type XII collagen gene in the peri-implant ligament. It was surprising to find that only the alveolar bone side of the peri-implant ligament showed the presence of type XII collagen, whereas the implant side of peri-implant ligament was completely devoid of this gene expression.[9] Although this model did not seem promising for future studies, the results led us to believe that the gene expression pattern near the implant surface could be totally different.

Dr John E. Davies of the University of Toronto then introduced us to his T-cell implant system. The T-shaped titanium housing with a hollow chamber allows bone tissue ingrowth when implanted in the rat femur. The tissue in the T-cell implant chamber provides reproducible tissue samples for gene expression studies that are relevant to the molecular biology of osseointegration.[5–7]

We then formulated the following hypothesis: "The presence of titanium implants with different surface topography modulates the expression of a specific set of genes that are not involved in osteotomy-induced bone wound healing; and these implant-induced gene products contribute to the establishment of ossointegration." This hypothesis was addressed by using the T-cell implant system and molecular biological protocols, thereby allowing comparative gene expression profiles.

Ogawa: Using differential display-polymerase chain reactions in the rat model, we isolated 3 genes (tentatively named

TO-1, TO-2, TO-3) that are differentially expressed when bone is deposited on the surface of titanium implants and not expressed during normal bone healing.[10] Differential expression of these genes was remarkable during the early stages of healing (up to week 2), and accelerated with acid-etched titanium surfaces compared with machined surfaces. We thus provided evidence that selected gene transcripts are induced or highly upregulated by titanium implants. We believe that exploring the function of these genes may provide novel clues to further understand the mechanisms of osseointegration.[11]

With respect to implant surfaces, what do you expect to see in the future?

Nishimura: In recent years, a new array of technologies has been developed, allowing fabrication and testing of nanoscale materials. Along with numerous other scientists, I believe that nanotechnology may significantly impact implant design in the future. Nanotechnology is defined by the US National Science and Technology Council's Nanoscale Science, Engineering and Technology subcommittee as follows: "Research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1–100 nanometer range, to provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices and systems that have novel properties and functions because of their small and/or intermediate size." A nanotechnology-based surface modification process may be developed with novel properties and functions. Recent studies highlight the profound influence of nanoscale topography or nanotextures on cell behaviors, suggesting that a new generation of nanotechnology implants may significantly advance our ability to control osseointegration. Our current and future studies will involve the development of nanotechnology-based implant surfaces in function, plus a detailed investigation of biological responses to implants with such surfaces.[12]

Ogawa: Since so-called second-generation implants with microlevel roughened surfaces have yielded very successful outcomes, the further improvement of implant surfaces for better osseointegration capacity seems to be a challenge. The concept our team developed was to improve the weakness and maintain the strength of the existing surfaces. One major barrier in implant biology is a biological dilemma of an inverted correlation between proliferation and differentiation rates of osteoblasts. For instance, microroughened titanium surfaces have advantages over the machined, relatively smooth surfaces in that they not only produce tissue-titanium mechanical interlocking, but also promote osteoblastic differentiation, resulting in faster bone formation. The bone mass, however, tends to be smaller than that around the machined surface. The increased differentiation associated with diminished proliferation of osteoblasts has been demonstrated in multiple culture studies on titanium surfaces.[13,14] If we can improve the disadvantage in osteoblastic proliferation while maintaining the advantages in osteoblastic differentiation, that would be a major breakthrough to raise the osseiontegration capability of titanium surfaces.

The emergence of nanotechnology's unique applications is already gaining traction in the surface technology and science of dental implants. Nanoscience includes bio- and chemomolecular manipulation and nanotopographical modification of implant surfaces, providing the tools, models, and technology platforms for implant biologic research and enabling us to explore the possibility of next-generation implants with improved osteoconductive capacity. Our team is committed to exploring and implementing nanostructuring technology in the development of new implant surfaces. Consequently, I organized and chaired a symposium entitled "Nanotechnology and Bone-Titanium Integration" at the 2002 IADR meeting. At the 2006 IADR meeting, I led the discussion in the symposium "Molecular- and Nano-Design of Dental Implants," which elicited provocative opinions regarding future applications of nanotechnology to implant surfaces; it certainly makes for interesting reading (see www.iadr.org for more information). One possible future strategy could be the addition of nanostructure to the existing microstructured surfaces to create a synergistic effect of molecular interlocking of the tissue with an established cellular affinity of the microtextured surface. We are clearly on the verge of an exciting new era in the world of dental and orthopedic implants.

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