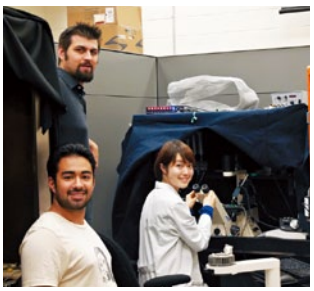
TOKYO MEDICAL AND DENTAL UNIVERSITY

TMDU

Research Activities 2017



TMDU—Committed to pioneering medical research

Contents

TMDU: Did you know...?

University Ranking by Subject

	Medicine	Dentistry
National Rank	4	1
World Rank	101-150	3

SOURCE: QS World University Ranking by Subject 2017

University Ranking by Number of Faculty / Students

Ranked #2 in Japan and #7 in the World

	Students	Faculty
Graduate	1,510	741
Undergraduate	1,489	

SOURCE: QS World University Ranking 2016

World's Best Small Universities

Ranked #1 in Japan and #17 in the World

SOURCE: Times Higher Education World's Best Small Universities 2017

University Hospitals Promoting Our Research

	Beds	Outpatients Per Year
Medical Hospital	763	581,993
Dental Hospital	60	450,710*

* Ranked #1 among dental hospitals in Japan

International Students

	No. of Int'l.Students	No. of Countries
Graduate Schools	277*	42

* Ranked #1 in enrollment among medical graduate schools in Japan

Message from the President

President

吉澤 靖之

Yasuyuki Yoshizawa

Tokyo Medical and Dental University (TMDU) was established in 1928 as the first national dental school in Japan. Since then, TMDU has grown into a comprehensive medical university by expanding into medicine in 1944 and nursing in 1951, and has become one of the most influential medical research institutions in the world. TMDU is currently located in the Ochanomizu / Yushima district in central Tokyo, which has been considered the traditional birthplace of scholarship in Japan since the 17th century.

We have approximately 3,000 students in our graduate and undergraduate schools, which include the Graduate School of Medical and Dental Sciences, Graduate School of Health Care Sciences, Faculty of Medicine, and Faculty of Dentistry. We foster our students under the TMDU Vision, "Cultivating professionals with knowledge and humanity, thereby contributing to people's well-being".

On our campus, we have two university hospitals, one for medicine and one for dentistry. The Medical Hospital is the most popular teaching hospital among medical interns in Japan and plays an important role nationally in clinical medicine. The Dental Hospital accepts the highest number of patients with oral disease in the country. The large number of patients visiting our hospitals gives us strong motivation and the opportunity for medical and dental research in order to understand and treat intractable diseases. For example, our research on regenerative medicine originates from clinical needs arising at these hospitals, as will be explained in detail in our Features of TMDU Research section. In addition, with an eye on the Tokyo 2020 Olympic and Paralympic Games, we have established a Sports Science Organization to provide integrated care for athletes and apply our scientific knowledge to public health. We have invited a gold medalist in the hammer throw at the 2004 Athens Olympics to serve as a professor in the Organization.

In addition to our schools and university hospitals, we have two research institutes, the Medical Research Institute and the Institute of Biomaterials and Bioengineering. Researchers there collaborate with industry to develop practical clinical applications for the benefit of society. In 2015, we also launched two venture companies involved in innovative medical instruments (endoscopic surgery robots) and drug development (heteroduplex oligonucleotides). We promote such translational research in order to link basic research to clinically applicable products. Furthermore, in 2016 we established the Center for Personalized Medicine for Healthy Aging to provide preventive medicine based on individual genetic backgrounds to contribute to longevity, and the Institute of Global Affairs / Institute of Education to promote university-wide globalization in the fields of research, education and medical treatment. We are also conducting collaborative investigations and education at our overseas research centers in Thailand, Ghana and Chile.

In this brochure, we highlight typical examples of state-of-the-art research activities at TMDU, which are in a continuous state of evolution and refinement. Although these activities represent only a fraction of the research underway at our university, I am nevertheless confident that these highlights will give you an idea of the exciting opportunities available here at TMDU for collaboration and study, open to researchers and students worldwide.

TMDU's research vision: To benefit humanity by bringing together the wisdom of diverse disciplines for pioneering research

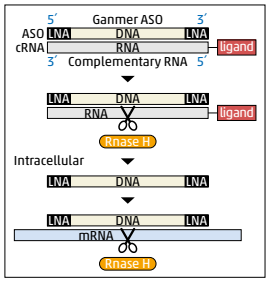
At Tokyo Medical and Dental University (TMDU), our goal as Japan's only comprehensive medical university goes beyond the production of impressive research results. We are committed to passing on those results in a way that benefits medical care and human health. In order to link our advanced and creative research and groundbreaking medical treatment technology to society at large, we focus not only on basic research but also on the social benefits it can bring. In other words, TMDU considers the key points to be how the results of basic research can be applied to clinical practice, how they can improve medical care, and how they can contribute to the health and well-being of society.

TMDU researchers belong to the Graduate School of Medical and Dental Sciences, the Graduate School of Health Care Sciences and two unique institutes, the Medical Research Institute and the Institute of Biomaterials and Bioengineering. At the same time, our researchers work in close association with many clinical departments and divisions of our two University Hospitals, the Medical Hospital and the Dental Hospital. TMDU has succeeded in forming many alliances between academia and industry to achieve research results that benefit patients. For example, recently, two venture companies have been established. One is Rena Therapeutics, which aims to bring heteronucleic acid technology to the practical level, aiming to deliver nucleic acid medicine to patients as quickly as possible through technical licenses to pharmaceutical companies and licensed drug candidates. The other is Riverfield, which has developed a new surgery support robot. In addition, we are promoting our international research collaboration. In fact, some 277 international students from 42 countries are currently learning at TMDU's graduate schools, which are the highest ranked among medical graduate schools in Japan.

In our brochure last year, *Research Activities 2016*, we introduced genome research and regenerative medicine as the fields of study to be emphasized. The theme this year is "research diversity," and our key areas of focus include tissue engineering, inflammation, autophagy, neurodegeneration, synovial stem cells, and more. In addition, we introduce professors researching biocompatible materials and sensors required to monitor the human body's status. Additionally, several top professors of Ophthalmology and Dentistry share their remarkable recent work, and four of our young researchers share the good results they have achieved in the face of pressure and difficulties.

We hope that this brochure will serve as a first step to inform readers about TMDU's broad and deep research.

TMDU Venture Businesses



Rena Therapeutics, Inc.

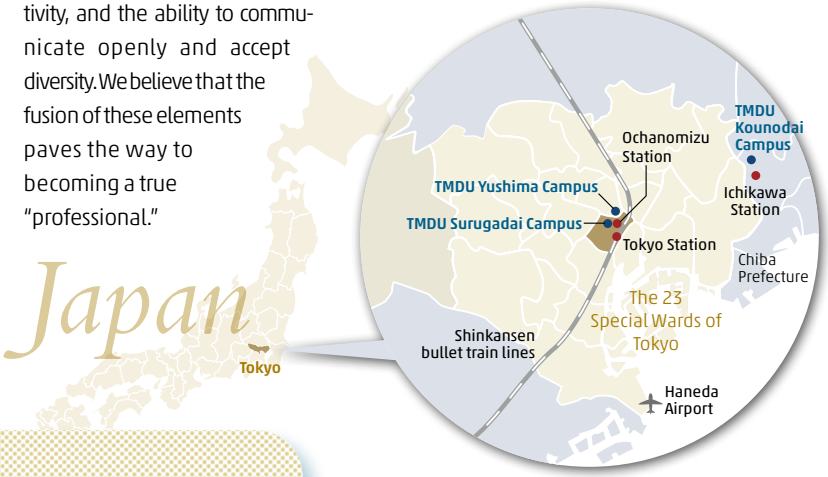
Establishment	January, 2015
Executives	**Founder & Chairman** Junichi Yano, PhD **President & CEO** Jun Sasaki **Founder & Director** Takashi Yamamoto **Founder & Scientific Adviser** Takanori Yokota, MD, PhD, TMDU
Business	Development of heteroduplex nucleic acid medicine
Mission	We address unmet medical needs by "Hetero-Duplex Oligonucleotide (HDO)," a novel class of nucleic acid medicine. HDO possesses a unique structure and allows effective drug delivery and chemical modifications.
Website	http://www.renatherapeutics.com/index.html

Riverfield, Inc.

Establishment	May, 2014
Executives	**CEO** Daisuke Haraguchi, PhD **Executive Director** Kenji Kawashima, PhD, TMDU **Executive Director** Junichi Sakata **Executive Director** Kotaro Tadano, PhD, Tokyo Tech
Business	Research and development of robotic surgical systems
Mission	To offer the global market high-quality medical devices based on reliable technology
Website	http://www.riverfieldinc.com/

Standing at the sacred birthplace of scholarship in Japan

Tokyo Medical and Dental University was established as a national medical and dental educational institution on October 12, 1928. Currently, TMDU is located in the Yushima / Shoheizaka area of Tokyo, which is considered sacred ground for scholarship and learning in Japan. As Japan's only comprehensive medical university and graduate school, TMDU has provided advanced medical treatment through a fusion of the medical and dental fields. It has worked to cultivate professionals with knowledge and humanity, thereby contributing to human health and the well-being of society. The "knowledge" referred to here includes learning, technology, and self-identity, while "humanity" means culture, sensitivity, and the ability to communicate openly and accept diversity. We believe that the fusion of these elements paves the way to becoming a true "professional."



TOKYO - The past and present

This landscape shows a view of Ochanomizu, where TMDU is located today. The buildings on the right-hand side, Yushima Seido and Shoheizaka School, were the center of scholarship since the 17th century, the Edo Period in Japan. Mt. Fuji can be seen in the far distance.

1800s

View of the Eastern Capital, Edo-Ochanomizu (woodblock by Shotei Hokuju)

1928

The Tokyo National School of Dentistry, the predecessor of TMDU, was established at Hitotsubashi.

Today, TMDU is still located in Ochanomizu / Yushima district where its predecessor, the Tokyo National School of Dentistry, had moved in 1930, two years after its founding. TMDU has become known as one of the most excellent research universities in Japan.

2017

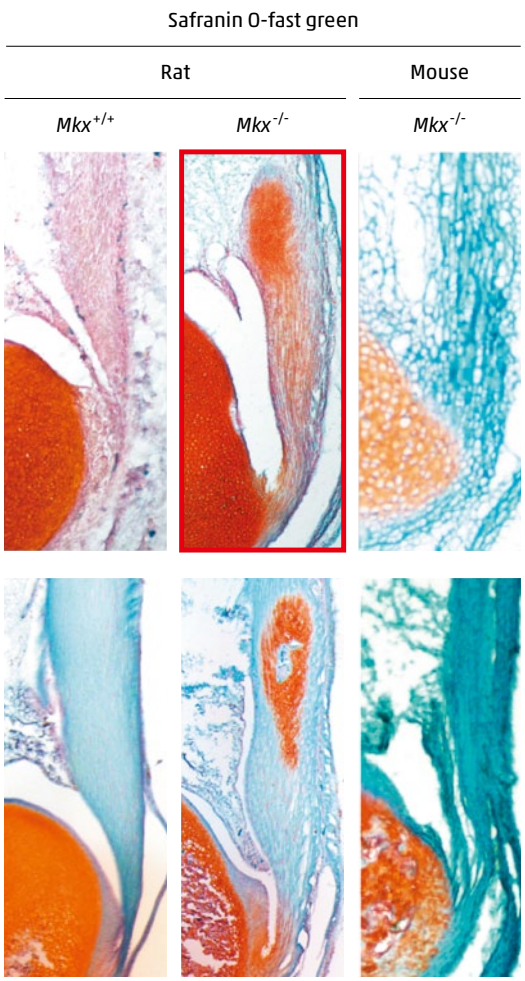
Present-day Ochanomizu, showing the same view as in the above woodblock. Ochanomizu Station is at the left and the TMDU Main Campus is at the right, with the Kanda River flowing between them.

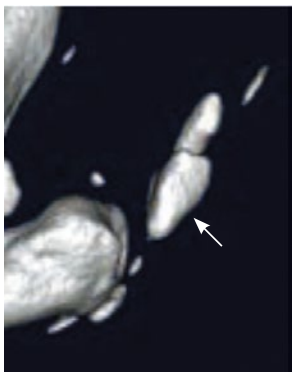
Mohawk transcription factor: A potential target for tissue engineering

Knockout rats represent a powerful animal model

Rats are the experimental animals of choice for several fields of research, including the musculoskeletal system, where smaller animals such as mice impose limitations ranging from an insufficient number of harvestable cells to difficulties evaluating the effects of mechanical load. Rats are also more physiologically similar to humans than mice are. However, it has proven to be technically challenging to derive genetically modified rats because of complications in isolating and maintaining embryonic stem (ES) cells.

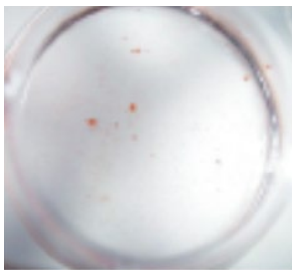
Heterotopic ossification in Achilles tendon of *Mkx*$^{-/-}$ rats



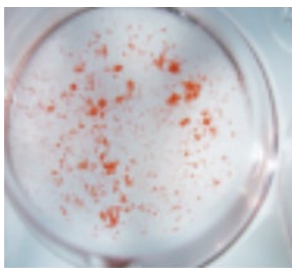


Micro-CT of the Achilles tendons of *Mkx*$^{-/-}$ rat

Mkx$^{+/+}$



Mkx$^{-/-}$



Mkx-deficiency accelerates osteogenic differentiation of tendon-derived cells

The development of genome editing technology for CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) has enabled precision genetic engineering to be achieved without ES cells. The Cas9 nuclease induces double-strand DNA breaks that are repaired by the cellular processes of homologous recombination or non-homologous endjoining. Further target specificity is achieved by the use of guide-RNA to direct nuclease activity.

Research by collaborators, coordinated at TMDU, used CRISPR/Cas9 technology to generate rats with a deletion of the gene encoding the Mohawk transcription factor (*Mkx*), which controls the expression of tendon-related genes.

Mkx plays an important role during tendon development

We derived three lines of F1 rats with targeted *Mkx* knockout, and in all three lines, we observed the distinctive 'wavy tail' phenotype and underdeveloped (hypoplasic) tendons previously seen in *Mkx*$^{-/-}$ mice. However, the tail phenotype was more pronounced in the *Mkx*$^{-/-}$ rats, which also showed the more severe phenotype of early heterotopic ossification of the Achilles tendon (the abnormal growth of bone within soft tissue). In humans, heterotopic ossification can be caused by injury to the soft tissue surrounding bones and joints, such as occurs during musculoskeletal trauma, excessive mechanical stress, or joint replacement, resulting in a painful condition that reduces the range of motion. Similarly, physiological assessment of the ankle joint during gait analysis of *Mkx* knockout rats showed reduced downward flexion of the foot compared with wild-type control *Mkx*$^{+/+}$ rats.

Molecular analysis of the *Mkx*$^{-/-}$ rats revealed the reduced expression of tendon-

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Hiroshi Asahara

Professor of Systems BioMedicine at TMDU

Dr. Asahara received his MD and PhD at Okayama University in 1997. He performed postdoctoral research at Harvard University, Salk Institute for Biological Studies and The Scripps Research Institute. He became Director at Japan's National Center for Child Health and Development in 2004. He joined TMDU as Professor of Systems BioMedicine in 2011.

related genes compared with the controls, which was accompanied by a corresponding reduced tensile strength of patellar tendons. Also, we observed elevated expression of genes associated with osteogenesis and chondrogenesis (bone and cartilage growth, respectively), which is indicative of heterotopic ossification. This was confirmed by micro-computed tomography. Meanwhile, transmission electron microscopy revealed smaller collagen fibril diameters in the tail tendons of *Mkx*$^{-/-}$ rats than in wild-type rats.

Mechanical stress prevents tendon ossification

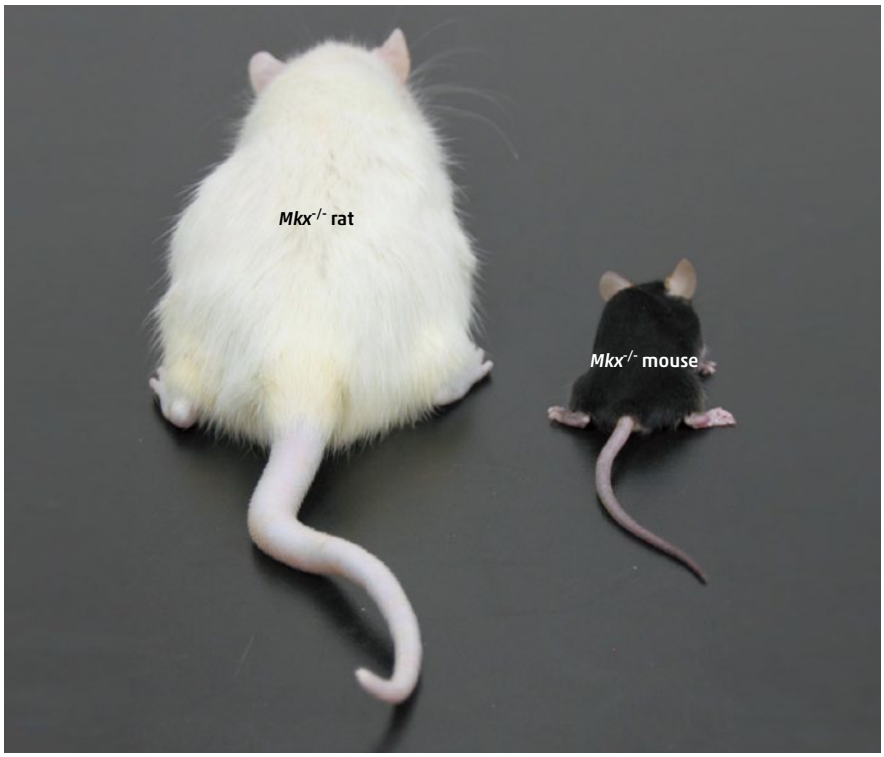
Abnormal tendon ossification in neonatal rats involves the development of cartilage within the tendon at birth, then the replacement of cartilage with bone. We found that in the absence of *Mkx,* tendon stem cells and progenitor cells that should differentiate into tendon cells instead become cartilage cells. Thus, tendon-derived *Mkx*$^{-/-}$ cells showed a tendency to undergo osteogenic and chondrogenic differentiation because absence of *Mkx* represses the tendon-related genes, such as extracellular matrix genes, and promotes the genes associated with chondrogenesis and osteogenesis. Conversely, *Mkx* overexpression reduced the differentiation of *Mkx*$^{-/-}$ cells into bone cells, cartilage cells, or adipocytes.

A lack of tendon cells derived from *Mkx*$^{-/-}$ mice has hampered previous attempts to perform a genome-wide search of *Mkx* targets. However, we obtained a sufficient volume of tendon cells from *Mkx*$^{-/-}$ rats to show that putative *Mkx* targets include both tendon-related genes (such as *Fmod*) and collagen genes, and also the *Sox* family of genes, which are associated with chondrogenic differentiation. *Fmod*-deficient mice have previously been shown to have heterotopic ossification of both the Achilles tendon and the knee joint.

Furthermore, because mechanical stress influences tendon development and promotes mesenchymal stem cells to differentiate into tendon cells, we investigated the effect of the loss of *Mkx* on the cellular response to mechanical stress. We stimulated *Mkx*$^{-/-}$ tendon-derived cells by mechanical stretching and observed increased expression of chondrogenic genes and enhanced differentiation into bone or cartilage cells. We surmise that the larger size of rats compared to mice causes greater mechanical stimulation of the tendon, resulting in increased chondrogenic differentiation and a more severe phenotype.

These findings suggest that *Mkx* controls the differentiation of tendon cells while simultaneously preventing their development into bone or cartilage. The introduction of *Mkx,* therefore, has potential as a novel repair mechanism for tendon damage or tissue engineering.

A white rat and black mouse with the characteristic wavy phenotypes in the tail associated with disruption of *Mkx*



Gene targeting of the transcription factor Mohawk in rats causes heterotopic ossification of Achilles tendon via failed tenogenesis
Proc. Natl. Acad. Sci., doi: 10.1073/pnas.1522054113

Basophil protease and allergic inflammation: Uncovering new links

Hajime Karasuyama

Executive Director at TMDU / Executive Vice President of University Innovation and Globalization / Professor of Immune Regulation

Q Please explain your findings on basophil responses in allergy.

A: I have been working with my TMDU colleagues to illustrate a novel role for mouse mast cell protease-11, or mMCP-11, originating from basophils, in triggering immunoglobulin E-mediated delayed onset allergic skin inflammation *in vivo*. Interestingly, while mMCP-11 deficiency had no impact on murine basophil development or homeostasis, the loss of mMCP-11 influenced allergic inflammation by decreasing the swelling of affected skin, lowering microvascular leakage and recruiting fewer leukocytes. Basophils likely migrate to regions of skin insult, are stimulated by allergens, and produce mMCP-11, which then recruits more leukocytes, including basophils. We showed that proteolytic mMCP-11 does not recruit leukocytes directly but instead triggers formation of cleaved products that then function as chemoattractants in a G protein-coupled receptor-mediated manner. Therefore, mMCP-11 represents an important effector molecule in allergic inflammation.

Q Mast cells and basophils share common features. Please comment on your focus on basophils.

A: Basophils mediate immune responses. While both basophils and mast cells contain proteases in intracellular granules that they release when activated, the profiles of proteases differ, implying exclusive functional roles. In addition to that, circulating basophils differ from tissue-localized mast cells and originate from distinct cell lineages. The functional activities of basophil proteases have not been fully understood, and this further motivates our study.

Recently, our research on the mast cell tryptase mMCP-11 found that it is predominantly produced by basophils rather than mast cells, despite its name. Separately, we have published a study reporting that basophils play non-redundant roles in murine immunoglobulin E-mediated delayed onset allergic skin inflammation. Our current research examined the molecular link between these phenomena.

Q How do your research interests align with the focus areas at TMDU?

A: There is a significant focus on medical research at TMDU and well-established facilities for supporting animal studies in research projects like ours. TMDU also emphasizes fostering academia–industry collaborations that promote translational medicine.

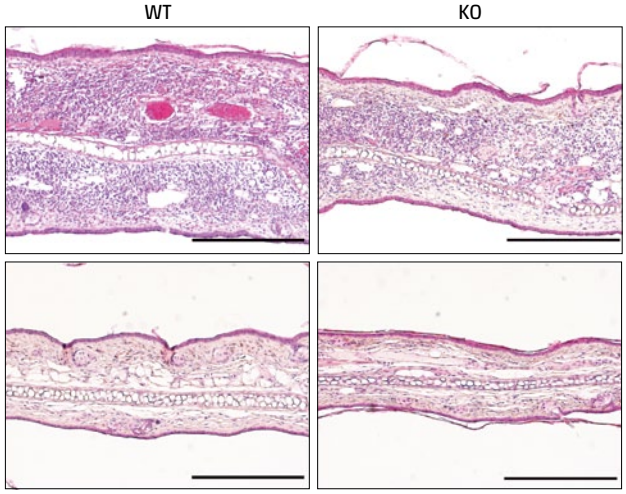
Q What are future directions for your research?

A: While our research takes significant steps toward better understanding basophil functional activity, the molecular mechanisms underlying inflammatory responses and leukocyte recruitment should be studied further. In particular, it is important to identify the target protein of mMCP-11 and explore the possible interaction of mMCP-11 with other molecules derived from leukocytes.

Q What are the therapeutic implications of your findings?

A: Our research illustrates new connections between basophil proteases and how they exacerbate inflammatory responses. Potential therapeutic strategies may target the chemoattractant roles of basophil protease products to mitigate allergic responses.

Hematoxylin and eosin-stained specimens of IgE-CAI (IgE-mediated chronic allergic inflammation) skin lesions on day 3 post-challenge



Histopathological examination of ear skin specimens indicated the reduction of cellular infiltration in the IgE-CAI skin lesion of mMCP-11-deficient (KO) mice compared to wild-type (WT) mice. Scale bars, 200 μm.

Modified from *Blood*, doi:10.1182/blood-2016-07-729392

Dr. Karasuyama graduated from the Faculty of Medicine at TMDU and completed his postgraduate work at the University of Tokyo, where he received his MD and PhD. He performed postdoctoral research at the Basel Institute for Immunology in Switzerland and the University of Tokyo. He became Head at the Tokyo Metropolitan Institute of Medical Science and joined TMDU as Professor and Chairman of the Department of Immune Regulation in 2000. He assumed the role of Executive Director and Executive Vice President in 2014.

Blood, doi: 10.1182/blood-2016-07-729392

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New imaging technique for faster and more accurate detection of cavities

Junji Tagami

Executive Director at TMDU / Executive Vice President of Education and International Student Exchange / Professor of Cariology and Operative Dentistry

Dr. Tagami completed his dental and graduate school at TMDU, where he received his DDS and PhD. He became Adjunct Assistant Professor at the Medical College of Georgia at Augusta University, and in 1994, assumed the post of Professor and Chairman of the Department of Operative Dentistry at Ohu University. He returned to TMDU in 1995 as Professor and Chairman of Cariology and Operative Dentistry. Dr. Tagami became Executive Director and Executive Vice President in 2014.

Q Your work has generated a significant technological advance in detecting cavities. Please tell us about this new technology.

A: The method we employ is called swept-source optical coherence tomography (SS-OCT), which uses high-speed frequency swept-laser light from a near-infrared laser. The light is projected at the occlusal surface of the tooth and scanned across its proximal surface. Two-dimensional images from below the tooth surface are generated by detecting the back-scattered laser beam signal, which is digitized over a time scale.

Q What methods are currently used to detect cavities, and how is the new method superior?

A: These days, oral health professionals typically use radiographs to detect cavities that are not visible to the naked eye. However, cavitated enamel lesions and dentin caries that comprise cavities can be difficult to detect on radiographic images, especially at the early stages. Our study has shown that images generated using SS-OCT are better than radiographic images for detecting cavities in several ways. First, cavities were detected at an earlier stage with SS-OCT than with radiographs. Also, SS-OCT detected cavities in areas that may be difficult to reach using radiographs. Finally, SS-OCT appears to provide more reliable and accurate images than bite-wing radiographs. In addition to these differences, SS-OCT images can be collected in real-time, enabling on-site diagnosis in a dental clinic.

Q How will the new method help patients with cavities?

A: Earlier detection of cavities using SS-OCT would allow the cavity to be treated when it is smaller, rather than detecting it later when the cavity could be larger and require more extensive and invasive treatment. This could reduce the pain experienced by the patient, which is associated with more complex dental procedures. Moreover, SS-OCT imaging does not require any radiation, so it can be used safely for dental diagnosis on patients such as pregnant woman and young children.

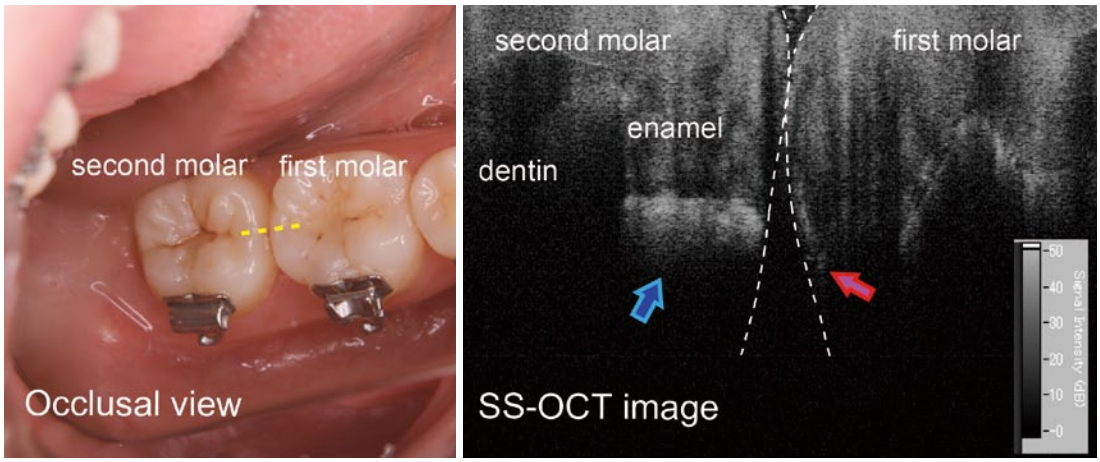
Q Tell us about any further improvements to the SS-OCT technique.

A: We already saw an improvement in cavity detection using two-dimensional imaging. Obtaining three-dimensional images in real time will further improve detection of early-stage cavities and cavities in areas that may be difficult to reach using X-rays. Our goal is to improve technology that can be used in dental offices, and this research is important in improving overall oral care.

J. Biophotonics, doi: 10.1002/jbio.201200210

Occlusal view (left) and SS-OCT image (right) of lower first and second molars

The SS-OCT image was obtained by scanning along the dotted yellow line shown in the left-hand image. SS-OCT reveals slight demineralization at the first molar (red arrow) and enamel caries at the second molar (blue arrow).



Synovial stem cells: Hope for cartilage and meniscus regeneration in osteoarthritis

Ichiro Sekiya

Director of the Center for Stem Cell and Regenerative Medicine
Professor of Applied Regenerative Medicine at TMDU

Q You work on the use of adult synovial stem cell transplants to achieve cartilage repair. What are the advantages of your method over existing therapies?

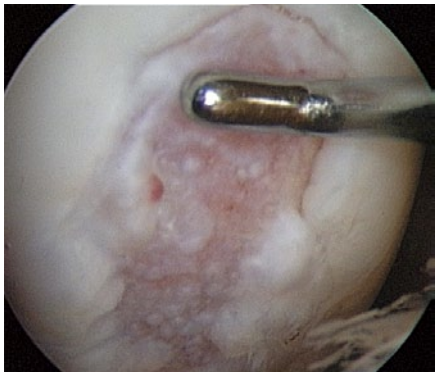
A: Current cartilage repair procedures include bone marrow stimulation to encourage new cartilage to form, but this is limited to small areas and the new cartilage structure may be poor. An alternative involves grafting small plugs of healthy cartilage from low weight-bearing areas of joints to damaged regions (mosaicplasty), but this is associated with donor site morbidity. Also, reports on implantation of a patient's own cartilage cells describe a loss of cartilage cell characteristics. These latter two techniques also involve open surgery, with its associated risks. I have worked with my colleagues at TMDU to devise a minimally invasive method, using a form of keyhole surgery. We harvest the soft tissue of a patient's joint (synovium), grow the stem cells derived from this, and then transfer them to the damaged area.

Q Can you describe the clinical procedure carried out in your study and the final outcome?

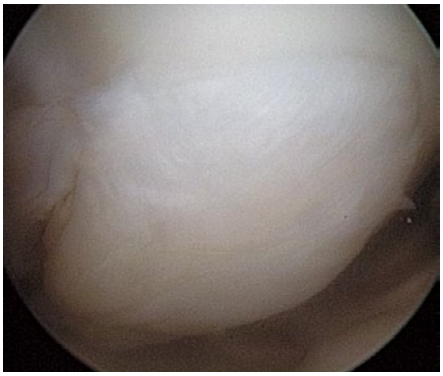
A: We enrolled 10 patients in the trial, all with a knee cartilage defect caused by trauma. We removed synovium from each patient and extracted mesenchymal stem cells (MSCs). We cultured these cells in each patient's own serum, and placed an average of 47 million stem cells in the damaged area using a needle, and kept the patient completely still for 10 minutes. The treatment was effective: MRI analysis showed repair of the area, and replacement cartilage was visible under a microscope. Orthopedic disability was scored and also significantly improved in all patients.

Arthroscopic transplantation of autologous synovial stem cells for cartilage defect

Cartilage defect

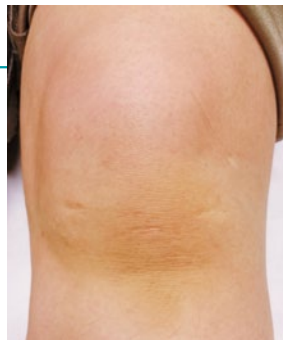


One year after transplantation



This treatment is effective and possible using small incisions.

Small incisions



Q Can you apply synovial MSCs to meniscus injuries?

A: The meniscus is a crescent-shaped fibrocartilagenous tissue in the knee and has a role in load distribution, stability, and lubrication. Symptomatic degenerative meniscus tears are common, and if surgery is required, arthroscopic partial meniscectomy, instead of meniscus repair, is generally selected due to the poor healing potential of meniscus, although meniscectomy is a cause of osteoarthritis. We performed another clinical study and found that transplantation of synovial MSCs was effective on repaired meniscus with degenerative tears.

Q Can you regenerate cartilage in osteoarthritis?

A: Knee osteoarthritis, characterized by pathological features including joint space narrowing, is a major public health issue causing chronic pain and disability in the elderly in most developed countries. We performed another clinical study of osteoarthritis in which synovial MSCs were transplanted to defective meniscus and cartilage after the centralization of extruded meniscus. Most patients showed improved clinical outcomes. In some types of osteoarthritis, cartilage can be regenerated with minimally invasive procedures and the transplantation of synovial MSCs.

Dr. Sekiya completed his medical and graduate school at TMDU, where he received his MD and PhD. He performed postdoctoral research in the United States at MCP Hahnemann University and Tulane University. Since 2013, he has been Director of the Center for Stem Cell and Regenerative Medicine and Professor of Applied Regenerative Medicine.

Clin. Orthop. Relat. Res., doi: 10.1007/s11999-015-4324-8 (2015)

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Three-dimensional MRI offers new insights into globe shape evaluation in ocular diseases

Kyoko Ohno-Matsui | Professor of Ophthalmology and Visual Science at TMDU

Dr. Ohno-Matsui graduated from the Faculty of Medicine at Yokohama City University, and received her MD and PhD at TMDU. She became Assistant Professor at TMDU in 1997, and Associate Professor in 2005. She took office as Professor in 2014 and leads the Department of Ophthalmology and Visual Science as Chairperson.

Q Please describe your findings on pathologic myopia and staphylomas.

A: Pathologic myopia causes blindness. With fellow researchers in Japan and around the world, we have demonstrated that diverse ocular shapes are linked to the advance of disease. We used three-dimensional magnetic resonance imaging (3D MRI) to examine 44 highly myopic patients and found that their eyes presented with symmetric nasal or posterior forms with barrel or cylindrical shapes, or were asymmetric and nasally or temporally misshapen, compared with normal, spherical emmetropic eyes. Characterizing ocular shape is critical for identifying preventive measures.

We also examined 105 patients to standardize definitions of posterior staphylomas in pathologic myopia. Panoramic Optos™ ophthalmoscopy fundus images, similar to conventional clinical images and infrared images, identified ocular staphyloma edges by irregularities in pigmentation, reflectance, and fluorescence. The 3D MRI results corresponded well with fundus images of abnormalities in patients with wide or narrow macular staphylomas, or inferior or peripapillary staphylomas. Patients without staphylomas could be accurately identified. Patients with staphylomas were older and suffered from worse vision than those without.

Q The classifications of myopia that you have proposed have been well received. Please elaborate.

A: Having global collaborators in places such as the United States, Australia, Singapore, and Europe, helps extend the work's international implications. With my colleagues, we have proposed international photographic classification systems for myopic maculopathy characterized by pathologic myopia, posterior staphylomas and posterior lesions. Our system of numerical grouping based on disease severity established a moderately uniform categorization of 100 images by inter- and intra-consensus.

Q How did you decide to focus on 3D MRI in your research?

A: Three-dimensional MRI renders holistic topographical images using volume-depicting techniques rapidly and non-invasively. Computerized analyses provide signal strength-based semi-automatic delineation of global boundaries. Using 3D MRI facilitates early identification and management of vision field flaws.

Q How do your research interests align with the focus areas at TMDU?

A: There is a significant focus on translational ophthalmological research at TMDU. I have worked in close association with the High Myopia Clinic at TMDU. Research examining possible associations between eye shape and orbital shape is also underway here.

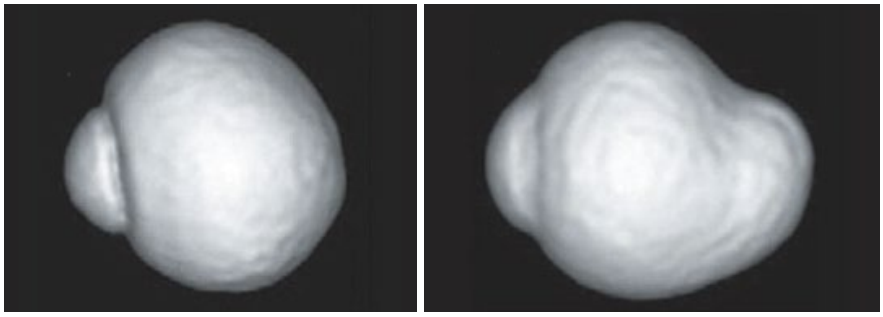
Q How do you anticipate future research progressing and what are the challenges?

A: Future research may include longitudinal studies to characterize shape alterations of human eyes with age, identifying the first signs of myopia and elucidating mechanisms underlying pathologic myopia. A challenge would be to find suitable animal models on which to study pathologic myopia.

Q What are the therapeutic implications of your findings?

A: Our findings will help elucidate underlying genetic factors in pathologic myopia and staphylomas. It is also especially relevant for establishing homogeneous classification methods for myopic maculopathy, given its high prevalence here in East Asia. The proposed classification systems will boost further studies in ocular disease.

3D MRI image of the eye (nasal view)



A normal emmetropic eye is almost spherical (left). An eye with pathologic myopia (right), where the globe is deformed and the posterior segment forms a protruding pouch (known as posterior staphyloma).

Ophthalmology, doi: 10.1016/j.ophtha.2011.01.018
Ophthalmology, doi: 10.1016/j.ophtha.2014.03.035
Am. J. Ophthalmol., doi: 10.1016/j.ajo.2015.01.022

Ultra-early Alzheimer's disease pathway offers preclinical therapy potential

Hitoshi Okazawa | Professor of Neuropathology at TMDU

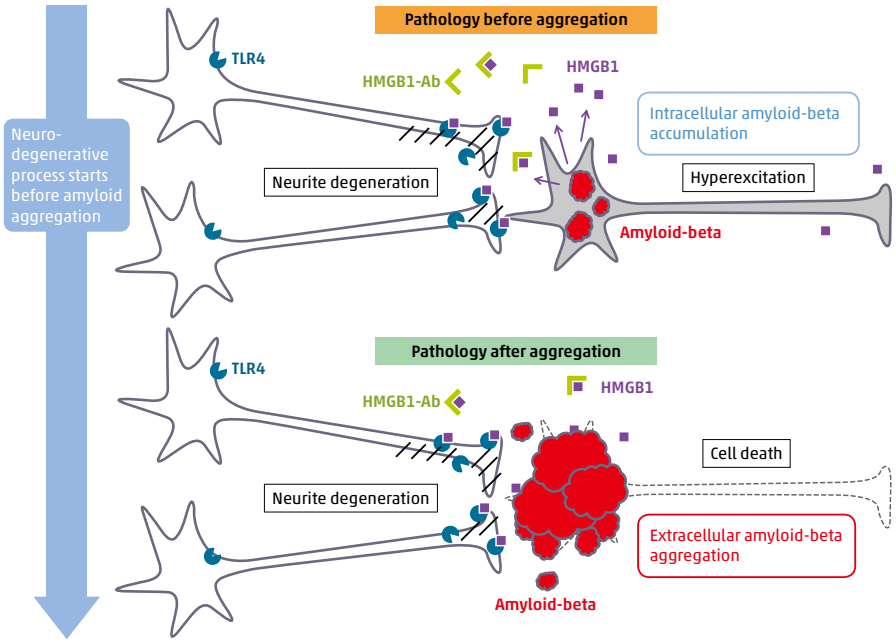
Q You focus on protein phosphorylation in the brains of Alzheimer's disease patients and mouse models. Please give us a brief overview of your latest findings.

A: In an earlier study, our team found that a protein called MARCKS was phosphorylated very early in mouse models, before the formation of amyloid plaques or cognitive impairment. The phosphorylation was also maintained in mouse models throughout the disease and detected even in autopsied brains of Alzheimer's disease patients. We noticed that over-stimulated or dying neurons released HMGB1 protein, which phosphorylated MARCKS via activation of TLR4 and downstream kinases. This led us to propose that HMGB1 is a promoter of an ultra-early phase (phase 0) pathology of Alzheimer's disease.

Q What made you focus on targeting the early stages of the disease?

A: Therapies that break down amyloid plaques have been shown to be ineffective in clinical trials, possibly because the plaques had already formed years before symptoms appeared. This means that early intervention or prevention are crucial to success, so we looked at molecular mechanisms that occur early on in the disease. Moreover, nobody has actually found the molecule that proves the existence of an ultra-early phase (phase 0) pathology of Alzheimer's disease before extracellular amyloid plaque (senile plaque) formation. MARCKS is the first case of such molecules directly indicating the existence of a phase 0 pathology of Alzheimer's disease.

HMGB1-antibody therapy targeting ultra-early phase (phase 0) pathology



Before amyloid aggregation, HMGB1 is released from neurons that accumulate intracellular amyloid-beta or that are hyper-excitatory. HMGB1 binds to TLR4 and activates MARCKS. The signal induces phosphorylation of MARCKS at Ser46, which leads to degeneration of neurites. Administration of anti-HMGB1 antibody prevents such pathology before amyloid aggregation.

Q Please elaborate the implications of your results.

A: We noticed that patients with the highest HMGB1 levels in their spinal fluid experienced particularly rapid progression of dementia. This finding suggests that HMGB1 could be a target for preclinical therapy. To test this, we developed an antibody against HMGB1, injected it into a mouse model before the onset of the disease, and found that it prevented cognitive impairment. This means that anti-HMGB1 antibody could delay the onset of Alzheimer's disease even when plaque accumulation has already started. This is very exciting because it indicates that people who will develop Alzheimer's disease in the future could be treated.

Q Do you have plans to expand on this work?

A: Naturally, we are now developing human antibodies against HGMB1 to be used in clinical trials. If everything goes smoothly, we will start trials in five years. Development of biomarkers is another plan. A high level of HMGB1 in spinal fluid may be linked to the quick development of dementia.

Dr. Okazawa graduated from the University of Tokyo, School of Medicine where he received his MD and PhD. He performed postdoctoral research at the Max-Planck Institute for Psychiatry in Munich as a staff scientist, and became Assistant Professor in the Department of Neurology at the University of Tokyo in 1994. In 2001, Dr. Okazawa became Head of the Tokyo Metropolitan Institute for Neuroscience, then joined TMDU as Professor in 2003. He became Director of the TMDU Center for Brain Integration Research in 2014.

Sci. Rep., doi: 10.1038/srep31895

Please Contact Us »» uraoffice.adm@tmd.ac.jp

Body cavity sensors offer monitoring solutions in daily medicine

Kohji Mitsubayashi | Professor of Biomedical Devices and Instrumentation at TMDU

Dr. Mitsubayashi received his PhD from the Department of Advanced Interdisciplinary Studies at the University of Tokyo in 1994. He became Associate Professor at Tokai University and in 1999, he joined the University of Perpignan in France as a visiting associate professor. Since 2003, he has been Professor at the Institute of Biomaterials and Bioengineering (IBB) at TMDU.

Q What prompted this informative review on biosensors?

A: We have made rapid strides in recent decades in biosensor fabrication, applications, and readouts. I wanted to write a systematic review of significant breakthroughs and challenges that remain, and to highlight promising avenues for further research and development. Biosensors are valuable tools that measure levels of biological factors in ocular or oral body cavities. They are wearable, non-invasive, long-term sensors that provide rapid, continuous quantification of physiologically relevant chemicals in perpetually present body fluids or gaseous exchanges. Human secretions, including urine, feces, perspiration, breath, nasal discharges, body odor, or even tears, can provide valuable snapshots of an individual's health, anxiety level, genetics, and immune status.

Q Please highlight key applications of biosensor technologies.

A: Body cavity sensors have changed medical monitoring. Transcutaneous oxygen sensors are user-friendly for preventing premature retinopathy in infants. Ocular telemetry sensors monitor glaucoma by correlating easily measurable corneal curvature as a readout for nocturnal variations and instabilities in ocular pressure. Tear glucose levels correlated to output current, which significantly corresponded with blood level, albeit with time delays and concentration differences. Interestingly, amperometric sensor-based contact lenses or modified commercial lenses were found to measure tear glucose and hypoxia reproducibly, mitigating interference confounders.

Salivary levels of risk factor uric acid in stress, gout or diabetes correlate with blood levels and are estimated non-invasively with electrode-based mouthguard platforms. Alternate applications include lactate measurement for fitness and sensors for measuring bacteria on teeth.

Q How have advances in fabrication technologies promoted biosensor development?

A: Microfluidics, MEMs and biomaterial-based technologies have revolutionized biosensor fabrication. Microsystems enable measurement of biological readouts in small volumes, reducing costs and increasing accessibility. Purpose- and microenvironment-specific biomaterial design with adaptable, biocompatible features have led to advances in polymer technologies with utilization of PEG, PTFE, or PDMS-DMA, the use of phospholipids MPC or PMEH, chemical coatings including gold or titania sol-gels, Nafion™ to reduce chemical interference, and even "smart" 3D reversible hydrogels.

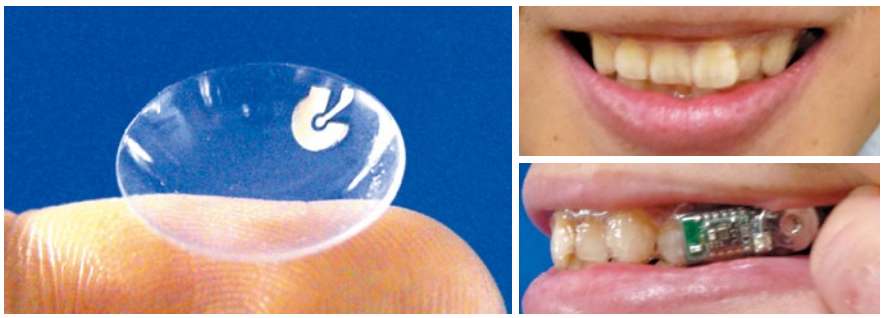
Q Please describe how your research fits in with TMDU's objectives?

A: My interests in developing biological materials and microsystem technologies are closely associated with biosensors. Body cavity sensors are convenient for the vulnerable aging population of Japan. For example, by quantifying flow, there are flexible, conductometric sensors that measure tear electrolyte concentrations and turnover rates, which can indicate tear gland malfunction in older patients with Keratoconjunctivitis sicca.

Q Please share your future targets and anticipated challenges.

A: While highly sensitive "biotransferrables" are gaining traction in the clinic, the next steps include making them less cytotoxic and more affordable for a broader range of applications.

Cavitas biosensors for non-invasive biomonitoring in daily medicine



Soft contact lens-type biosensor with biocompatible polymers for tear glucose monitoring (left); Mouthguard telemetric sensor (right: front & side views) with Bluetooth 4.0 LE (low energy) transmitter

Electroanalysis, doi: 10.1002/elan.201600083

Alternative autophagy pathways conserved from yeast to mammals

Shigeomi Shimizu | Professor of Pathological Cell Biology at TMDU

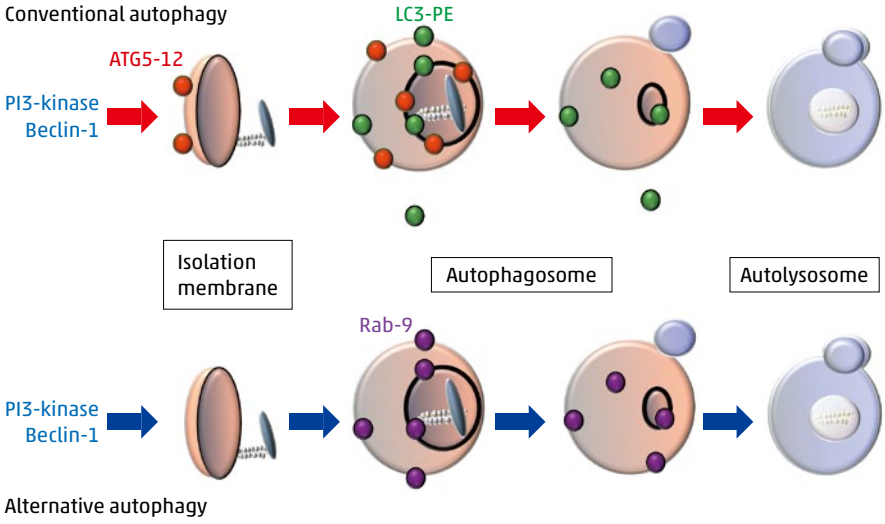
Q Can you explain the importance of autophagy to a cell?

A: Autophagy involves the breakdown of unwanted or damaged cellular contents through digestion within autolysosomes using lysosomal hydrolytic enzymes. It is a protective mechanism that is activated when the cell is stressed by damage to DNA or organelles, or following nutrient starvation. In addition to removing damaged proteins, or even whole organelles, it can also help to regulate protein expression levels by low-level constitutive autophagy.

Q You have identified an alternative autophagy process. How does this differ from the conventional system?

A: Autophagy occurs through the actions of more than 30 proteins, including Atg5 and Atg7, which are highly conserved from yeast to mammals. However, together with my many outstanding colleagues at TMDU and other fine Japanese institutions, we noticed that mammalian cells lacking Atg5 and Atg7 were healthy and still able to undergo autophagy-mediated protein degradation, controlled by Ulk1 protein rather than Atg5/Atg7. This happened when the cells were severely stressed by DNA damage rather than nutrient deprivation. Although the structures involved in this alternative autophagy exactly resemble those of conventional autophagy, they have different functions and can degrade different subcellular components. For example, in the maturation process of red blood cells, reticulocytes lose organelles such as mitochondria to become erythrocytes (the mature cell). My research teams have shown that this mitochondrial clearance was mediated by Ulk1-dependent alternative autophagy, particularly in fetal rather than adult reticulocytes.

Schematic model of autophagy



There are at least two modes of autophagy, *i.e.* conventional and alternative autophagy. Conventional autophagy depends on Atg5 and Atg12 and is associated with LC3 modification. In contrast, alternative autophagy occurs independent of Atg5 expression and LC3 modification, but depends on Rab9. Although both these processes lead to bulk degradation of damaged proteins or organelles by generating autolysosomes, they seem to be activated by different stimuli, in different cell types and have different physiological roles.

Q What are the implications of your findings for cell biology and beyond?

A: Our work shows the existence of an alternative protein degradation pathway that can compensate for Atg5/Atg7-dependent autophagy. More recently, we found a similar Atg5/Atg7-independent autophagy in yeast, which is activated when the movement of cargo from the Golgi to the plasma membrane is disrupted. This pathway uses Golgi-mediated structures to enclose the material to be degraded. Just like conventional autophagy, we showed that it is phylogenetically conserved in mammals, suggesting its evolutionary importance. We have been able to identify the yeast genes required for this process and find their mammalian equivalents.

Q What future plans do you have to increase our understanding of this field?

A: We have generated mice lacking the expression of genes that control alternative autophagy. The next step is to explore the molecular mechanisms that control this process and identify their physiological relevance.

Dr. Shimizu graduated from the Faculty of Medicine at Osaka University, where he received his MD and PhD. He became Assistant Professor (1995) and Associate Professor (2000) at Osaka University. He joined TMDU as Professor of Pathological Cell Biology at Medical Research Institute in 2006.

Nature, doi: 10.1038/nature08455
Nat. Commun., doi: 10.1038/ncomms5004
EMBO J., doi: 10.15252/embj.201593191

High pressure tissue decellularization offers promise for tissue engineering applications

Akio Kishida | Professor of Material-based Medical Engineering at TMDU

Dr. Kishida graduated from the Faculty of Engineering at Kyoto University, where he received his MD and PhD. He became Assistant Professor in 1992, and Associate Professor in 1994 at Kagoshima University. He joined TMDU as Professor of Material-based Medical Engineering at the Institute of Biomaterials and Bioengineering in 2004. In 2014, TMDU established the Department of Acellular Biomaterials and Regenerative Medicine and Dr. Kishida became the field manager there.

Q Your work involves decellularized tissues. What does this mean, and why is it important for the field of tissue engineering?

A: From the late 1990s, it has been reported that human decellularized tissue-extracellular matrix from which cellular components have been removed-works well as a substitute for living tissue. Decellularized tissue exhibits good biocompatibility, and the lack of donor cellular material minimizes the risk of graft rejection. Importantly, the removal of donor cellular material may allow for the use of animal tissue as a source for xenotransplantation into humans, so this technology has the potential to provide a high-performance scaffold for tissue engineering.

Q Could you explain the technique you have developed, and any challenges you had to overcome?

A: The main existing approach for decellularizing tissues involves using detergents. Such techniques may leave residual cytotoxic chemicals or change the mechanical properties of the tissue. We developed an alternative method of decellularizing tissues using high-hydrostatic pressure (HHP, >600 MPa). The cell debris can then be removed by washing, without chemicals or detergents.

Q What are the benefits of this method of decellularization?

A: *In vivo* grafts of HHP-decellularized tissues show minimal inflammation and exhibit good long-term stability. Grafted decellularized tissues can also strongly recruit host cells, facilitating rapid integration into host tissue. An additional benefit is that HHP has a sterilizing effect: Because the treatment disrupts lipid bilayers, it can destroy pathogens such as bacteria, fungi, and some viruses.

Q What kinds of applications for these decellularized tissues have you explored?

A: We transplanted decellularized porcine corneas into rabbits, and achieved transparent corneas that lasted for at least twelve months after transplantation.

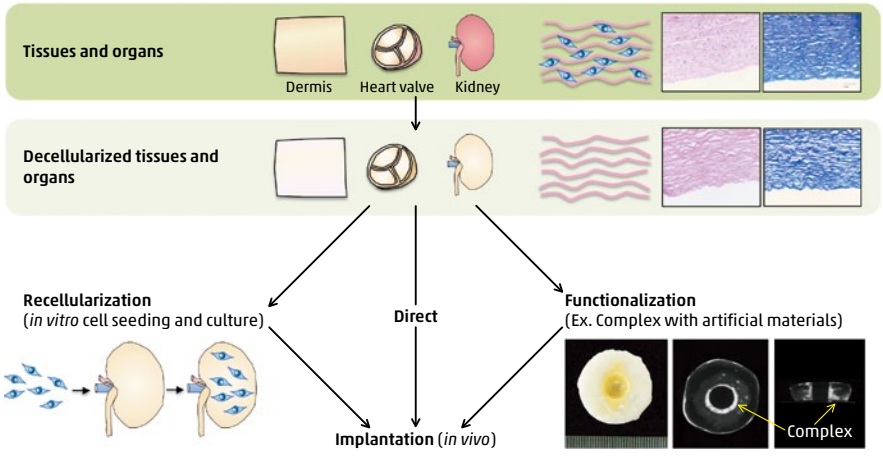
Another challenge we tackled was developing graft materials that can function as an interface between hard and soft tissues. We have used decellularized tissues in conjunction with a synthetic polymer to develop a hybrid biomaterial. This material can function as an interface between different tissue types, or between a tissue and an implanted synthetic device.

We also explored using powders made from decellularized tissues for treating myocardial infarction in a rat model. Decellularized liver powder promoted cell integration and neovascularization both *in vitro* and *in vivo*, and suppressed myocardial necrosis.

Q How do you expect your research to develop in the future?

A: We are working on regenerating bone marrow, brain, and cartilage using the relevant decellularized tissues. Decellularized tissue is emerging as superior to many artificial materials currently used in tissue engineering. We will continue working with our research partners to explore the potential of decellularized tissue.

Overview of applications of decellularized tissues and organs



Mater. Sci. Eng. C, doi: 10.1016/j.msec.2013.11.007
Adv. Sci. Tech., doi: 10.4028/www.scientific.net/AST.76.125
Biomaterials, doi: 10.1016/j.biomaterials.2010.01.073
Biomaterials, doi: 10.1016/j.biomaterials.2010.01.122
Mater. Sci. Eng. C, doi: 10.1016/j.msec.2015.07.010

Transistor-based sensing system finds ion leakage in cells injured by nanomaterials

Tatsuro Goda

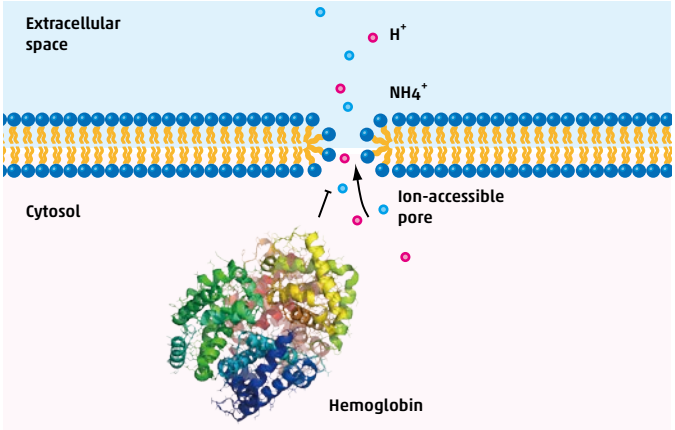
Assistant Professor of Bioelectronics at TMDU

Cytoplasmic delivery of nanomaterials with high efficiency and low cytotoxicity is of interest for nanomedicine. Studies on viruses have identified a series of oligopeptides suitable for efficient cytoplasmic invasion. Some nanomaterials can enter cells without endocytosis. In earlier research, we found passive diffusion of amphipathic polymers that mimic phospholipids, across the membranes of live cells (*Biomaterials* doi: 10.1016/j.biomaterials.2009.11.095). However, improvement of spatiotemporal resolution in analytical methods is necessary to elucidate the underlying mechanisms of this phenomenon.

Prompted by this, we developed an active pH-sensing system based on the fact that the proton is the smallest molecule, but it can only permeate through damaged cell membranes. A silicon-based transistor for label-free pH-sensing is amenable to downsizing and integration into a small chip, enabling high-throughput single cell analysis. To evaluate ion leakage across the plasma membranes, we injected ammonium chloride into a solution of cells that had been cultured onto the transistor. When we exposed the cells to nanomaterials, we observed leakages of H^+ and NH_4^+ but no leakage of hemoglobin. (*Acta Biomater.*, doi: 10.1016/j.actbio.2016.12.018). We understood that this was possible because the ions are so much smaller than hemoglobin (<0.33 nm vs >3.1 nm). Experiments over an extended period distinguished the ion leakage induced by cell apoptosis from that caused by membrane lysis by invading compounds. Comparison with conventional cytotoxicity assays classified a form of cell death. Our pH-sensing method is fast and sensitive, and is useful for understanding interactions between cells and nanomaterials.

I graduated from Kyoto University in 2003 and received my PhD (summa cum laude) in Material Engineering from the University of Tokyo in 2008. After a year on a JSPS postdoctoral fellowship in Tokyo, I began researching biosensors and bioelectronics at the National Institute of Materials Sciences in Tsukuba. In 2010 I became Assistant Professor at the Institute of Biomaterials and Bioengineering at TMDU. Since then, I have also been a guest researcher at Nanyang Technological University in Singapore and at Karolinska Institute in Stockholm.

Detection of small pores on plasma membrane by observing ion leakage



Prevention of knee osteoarthritis: From reducing injury to restoring function

Hideyuki Koga

Junior Associate Professor of Joint Surgery and Sports Medicine at TMDU

A major challenge in the field of orthopedics is to prevent osteoarthritis (OA) of the knee. OA can be induced by several factors, and secondary OA after traumatic events such as ligament or meniscus injuries should be prevented by various interventions.

After having defended my PhD at TMDU in 2008, I worked at the Oslo Sports Trauma Research Center in Norway from 2008 until 2010. My research concerned prevention of the knee's anterior cruciate ligament (ACL) injury. The ACL injury is the most frequent sports-related injury that requires surgery, and OA after ACL injuries has been a big issue. Therefore, a detailed description of the injury mechanism has been needed to develop ACL injury prevention measures. This groundbreaking research clarified, for the first time, ever detailed ACL injury mechanisms using a sophisticated 3D-video analysis called the "model-based image-matching technique" (*Am. J. Sports Med.*, doi: 10.1177/0363546510373570). I am still working on ACL injury prevention, especially focusing on ski injuries as a member of the medical committee of the International Ski Federation.

Since returning to TMDU in 2010, I have been performing many knee ligament and meniscus surgeries as a surgeon. Concurrently, I have been conducting clinical and biomechanical research regarding such surgeries.

Knee instability caused by ACL injury can be categorized into two types, anterior instability and rotatory instability. Traditional ACL reconstruction was considered successful in restoring anterior stability, although some patients showed residual rotatory instability, which correlated with worsening outcomes and development of secondary OA. I tried to figure out intraoperative factors that affect rotatory instability from a biomechanical perspective, and clarified optimal settings for ACL reconstruction (*Am. J. Sports Med.*, doi:10.1177/0363546511426696; *Am. J. Sports Med.*, doi:10.1177/0363546514567069).

The other area I am pursuing energetically is meniscus preservation. The meniscus plays an important role in protecting articular cartilage through absorbing joint-loading. Extrusion of the meniscus suggests a failure of the load-absorbing function, and is correlated with progression of OA. Until now, however, there has been no effective surgical procedure for meniscus extrusion. I have developed a new surgical procedure called arthroscopic centralization to reduce and prevent meniscus extrusion. I have just reported excellent 2-year clinical results (*Arthroscopy*, doi:10.1016/j.arthro.2016.01.052). Currently, I am applying this technique to OA of the knee to restore knee function, and hopefully, prevent progression of OA and reduce the number of patients with severe OA who require knee arthroplasty.

Analysis of ACL injury mechanisms by the sophisticated "model-based image-matching technique"

Study of neurodegenerative diseases and cell death *in vivo* finds new signaling pathway

Chen Xigui

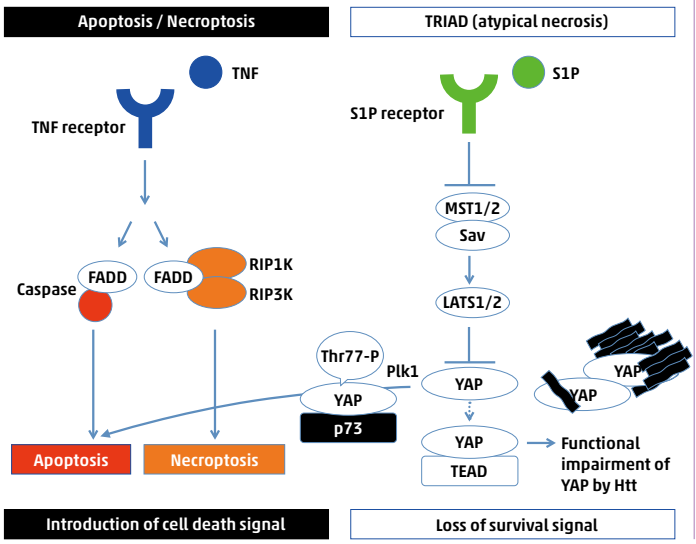
Specially Appointed Assistant Professor of Neuropathology at TMDU

After four years as a postdoctoral fellow at the University of Tokyo working on research into neuron synapse formation, I started my career in the Department of Neuropathology at TMDU. Our lab's theme is the elucidation of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Huntington's disease (HD).

Neurodegenerative diseases are characterized by slow progression compared with neuronal dysfunction and cell death. The typical cell deaths are apoptosis, necrosis and autophagic cell death. In 2006, our lab identified a new subtype of necrotic cell death named TRIAD (transcriptional repression-induced atypical cell death). In our research, we have captured images of TRIAD in the brain of live HD model mice. Through two-photon microscopy, we were able to observe the endoplasmic reticulum (ER) of neurons in the brain. In mutant HD-model mice, the ER enlarges, and the cell body forms an asymmetrical balloon shape until, finally, it ruptures. Pharmacological and genetic analyses revealed that this subtype of necrotic cell death is distinct from the RIP1/3 pathway-dependent necroptosis, as it is mediated by a functional deficiency of TEAD/YAP-dependent transcription.

In addition, we found that a cell cycle regulator, Plk1, switches the balance between TEAD/YAP-dependent necrosis and p73/YAP-dependent apoptosis by shifting the interaction partner of YAP from TEAD to p73 through YAP phosphorylation at Thr77. *In vivo* imaging of ER with two-photon microscopy detected the ER enlargement. Viral vector-mediated delivery of YAP, as well as chemical inhibitors of the Hippo pathway, such as S1P, repairs the ER instability and necrosis in HD model mice. Intriguingly, S1P completely stopped the decline of motor function in HD model mice even after the onset of symptoms. Collectively, we suggest that targeting the signaling pathway of TRIAD (TEAD/YAP-transcription-dependent necrosis) could lead to a therapeutic development to fight HD.

TRIAD signal pathway



Childcare leave and supportive colleagues make high-level research possible for mothers

Mariko Negi

Assistant Professor of Human Pathology at TMDU

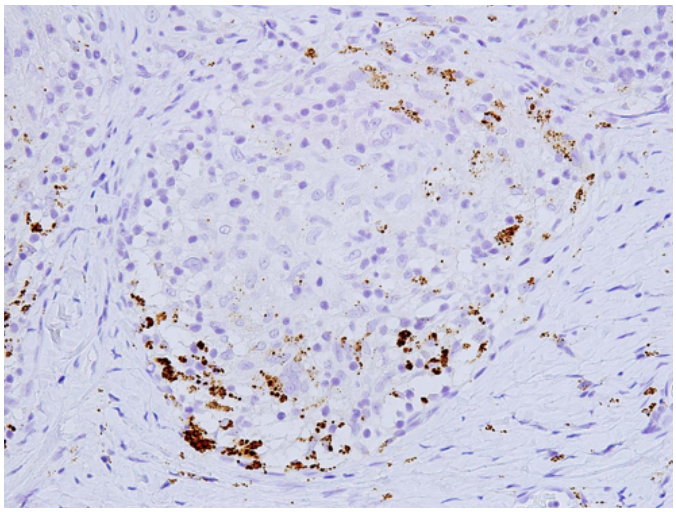
I started my career as a pathologist at TMDU after I graduated from medical school and completed clinical training. During graduate school, I was engaged in research to prove the hypothesis that the onset of sarcoidosis, which is a granulomatous disorder of unknown etiology that affects multiple organs, is caused by a bacterium resident on the skin, *Propionibacterium acnes*. Our team investigated novel monoclonal antibodies that were specific to *P. acnes*. We published data showing that these antibodies were present more frequently in sarcoidosis granuloma and its surrounding tissues compared to the tissues of other diseases (*Mod. Pathol.*, doi: 10.1038/modpathol.2012.80).

Since graduating from graduate school, I have been working as an assistant professor of human pathology at TMDU, doing research, teaching medical students, conducting daily pathological diagnoses, and attending conferences with clinicians in the university-affiliated hospital. In particular, I have been involved in joint conferences with the gastroenterology department that focus on difficult cases of inflammatory bowel disease, of which there are an abundant number of cases in our hospital. As a pathologist, I find this a challenging task.

In my personal life, I have been raising our two children while working as an assistant professor. I was able to acquire a childcare leave thanks to the great cooperation of my department. Although I was worried that it would prove difficult to retain the diagnostic skills of a pathologist while on leave, thanks to my colleagues' support, I was able to return quickly to daily work and retain my skills. As a result of raising children, I am not able to spend as many hours at work as before, but I find I concentrate intensely while in the lab in order to complete the same density of work.

These days, the number of female pathologists in Japan is increasing, even in our department. I will certainly help my juniors in the future if they choose to pursue both paths – as a pathologist and as a mother – the way my seniors did for me.

P. acnes within sarcoid granulomas



Immunostaining with PAB antibodies specific to *P. acnes*.

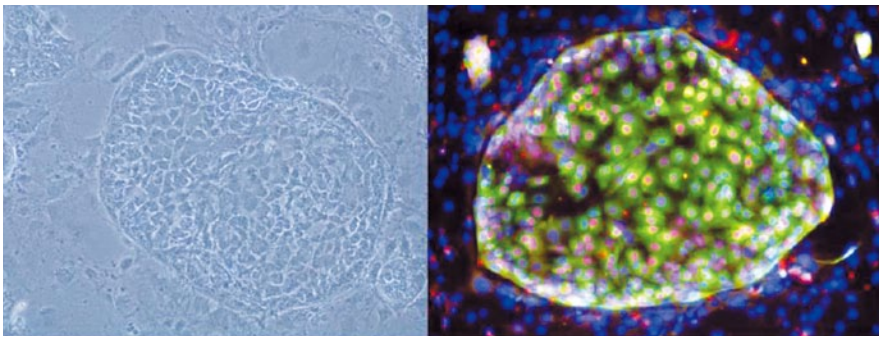
New stem cell-derived liver cell lines provide useful models of persistent hepatitis B infection

Progress in research on hepatitis B has been impeded by the lack of cell models appropriate to study the mechanisms by which the virus persists intracellularly. TMDU-led researchers have now overcome this problem by developing two hepatocyte cell lines derived from induced pluripotent stem (iPS) cells. These cell lines mirror features of liver cells infected *in vivo* such as long-term infection without major phenotypic changes and the mass production of infectious viral particles.

The research team led by Professor Yasuhiro Asahina found that the less differentiated of these two cell lines, immature proliferating hepatic progenitor-like cells, was particularly promising. When the cells overexpressed NTCP (sodium taurocholate cotransporting polypeptide, the site of hepatitis B virus cell entry), they became highly infected, could be stably cultured, and exhibited typical virus-induced immune responses. These cells should be useful tools for characterizing viral infection, identifying new target molecules, and testing new drugs in the fight against chronic hepatitis B infection.

Sci. Rep., doi: 10.1038/srep29358

iPS cell-derived hepatic progenitor-like cells



Cells in CD13+CD133+ fraction can form large colonies expressing HN4Fα (red) and AFP (green). They were stably cultured on feeder cells for more than three months.

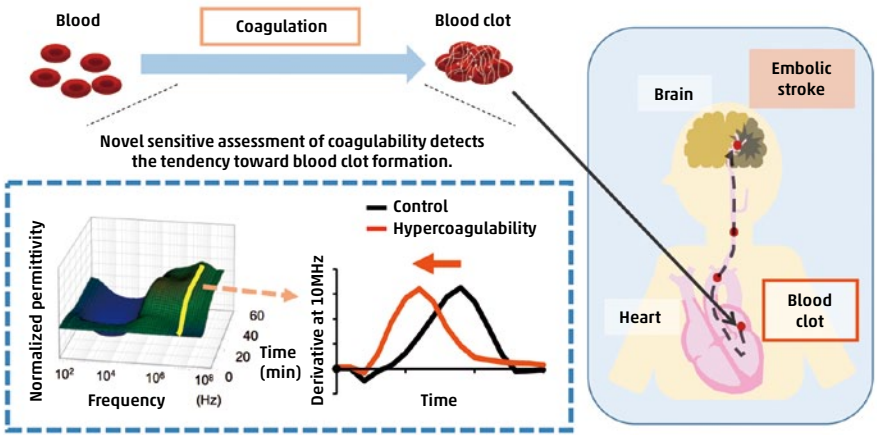
Novel coagulation test detects embolic stroke risk

The $CHADS_2$ scoring system, which was developed to estimate stroke risk in patients with atrial fibrillation, is also beneficial for individuals without abnormal rhythms, and high scores are associated with an increased tendency for blood to coagulate. However, the lack of a sensitive method to evaluate blood clotting has prevented this association from being fully investigated.

Associate Professor Tetsuo Sasano led a TMDU research team in applying the recently developed dielectric blood coagulometry test, which measures coagulability, and found it to be extremely sensitive in detecting small changes over time in the clotting ability of whole blood. The test enabled the calculation of a highly reducible and reliable coagulation index from blood that had been artificially thinned using heparin, or clotted using tissue factor. This index correlated with the $CHADS_2$ score and moreover, provided additional quantitative information about thrombosis risk. It also demonstrated the anticoagulative effects of drugs such as warfarin, indicating its utility for monitoring the efficacy and safety of anticoagulation therapy.

PLoS One, doi: 10.1371/journal.pone.0156557

The sensitive assessment of blood coagulability



Blood clots, generated by the coagulation of blood, can evoke an embolic stroke. The novel sensitive assessment of coagulability could detect the tendency toward blood clot formation by measuring the change in dielectric permittivity of the whole blood. The less time needed to change the permittivity, the greater the hypercoagulability, resulting in a higher risk of stroke.

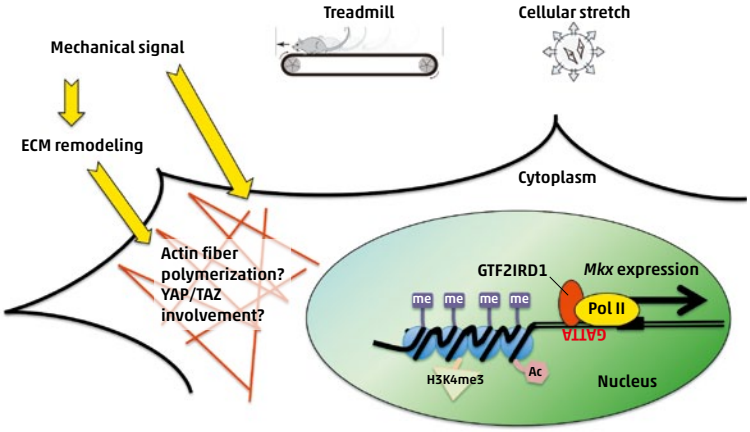
Tendon cell transcription factors key to sensing and responding to mechanical forces

Cells of mammalian tendons respond to environmental physical forces by remodeling their extracellular matrix in order to provide resistance to mechanical stress. The transcription factor Mohawk (*Mkx*) is involved in tendon development, but no *Mkx* upstream regulators have yet been identified, and the molecular mechanisms by which tendon cells receive mechanical signals are unknown.

Professor Hiroshi Asahara and colleagues centered at TMDU led research to show that *Mkx* was stimulated by physical exercise or cellular stretching to regulate downstream genes such as proteoglycans and type I collagens, resulting in increased collagen fiber thickening. The transcription factor GTF2IRD1, a candidate Mkx upstream gene identified by functional screening, is usually expressed in the tendon cell cytoplasm. However, under mechanical stretching, it was found to translocate to the nucleus, where it bound to the *Mkx* promoter region to induce *Mkx* transcription through histone modification. These findings about the *Mkx* transcriptional network and its role in mechano-sensing have important implications for tendon repair and future uses of Mkx as a therapeutic target for regenerative medicine.

Mol. Cell. Biol., doi: 10.1128/MCB.00950-15

Mechanism of mechanotransduction



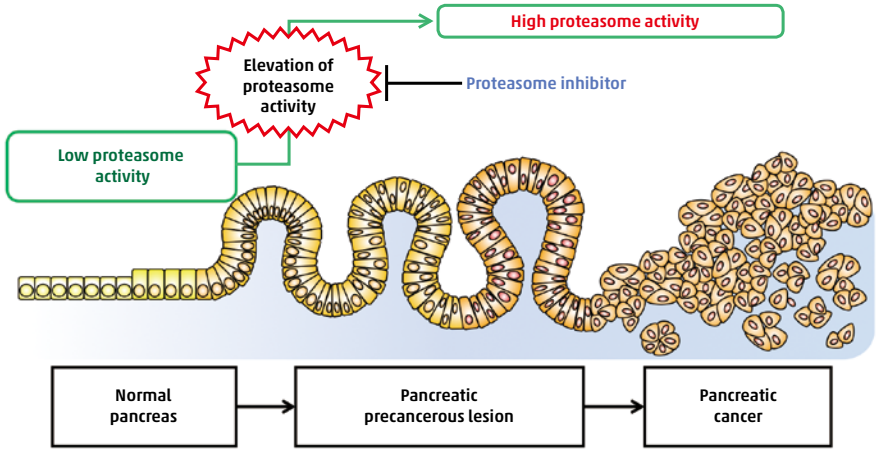
Mechanical signaling by treadmill exercise *in vivo*, or by cellular stretching *in vitro*, induces translocation of GTF2IRD1 to the nucleus to promote *Mkx* expression.

Proteasome involvement discovered in initial stage of pancreatic cancer

The rapid growth of cancer cells is known to require an increased activity and turnover of proteins, in which a protein-degrading component, the proteasome, is involved. However, it has remained unclear whether the proteasome is required only for the maintenance of existing cancer cells, or also for the emergence of a cancerous lesion. By establishing a transgenic mouse model that was prone to developing pancreatic lesions and expressed a marker for proteasome activity, a team of researchers led by TMDU showed that such activity is indeed essential for the formation of precancerous pancreatic lesions.

Using this mouse model, Professor Shinji Tanaka and colleagues found that an inhibitor of proteasome activity reduced the formation of pancreatic lesions but did not affect ones that already had been formed. Given the high mortality of pancreatic cancer patients because the disease typically is not recognized until at a late stage, the discovery of proteasome involvement during cancer initiation could provide useful markers for earlier diagnosis and improved prognosis of this disease.

The critical role of proteasome in pancreatic carcinogenesis



A normal pancreas has intrinsically low proteasome activity. Dramatic elevation of proteasome activity is an initial and essential step to forming precancerous lesions, which lead to pancreatic cancer.

Sci. Rep., doi: 10.1038/srep27044

Highlights of Recent Notable Publications

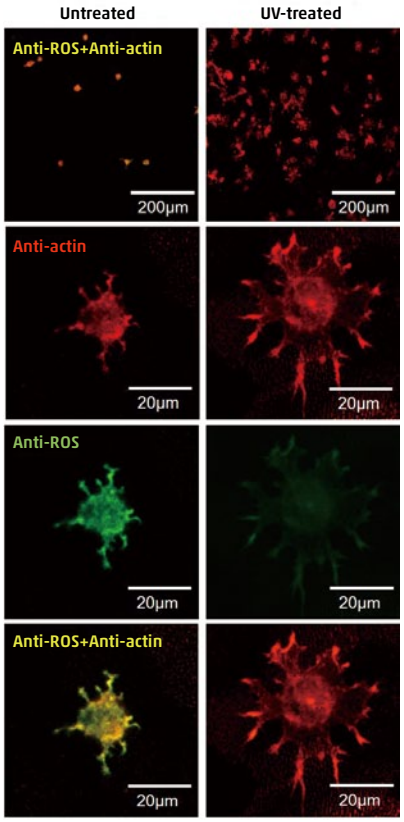
A novel antioxidant mechanism for ultraviolet light-induced implant biocompatibility

Bone-anchored implants are an important orthopedic and dental treatment. When cells come into contact with implant biomaterials, they produce more reactive oxygen species (ROS), and ROS overproduction can lead to oxidative stress. This reduces the proliferation and bone-forming capability of osteoblasts. Treating titanium implants with ultraviolet (UV) light increases hydrophilicity and removes hydrocarbons on the titanium surface. This improves cellular compatibility and bone-implant integration. However, how UV treatment affects ROS production in cells exposed to titanium implants remains unknown.

TMDU's Junior Associate Professor Takeshi Ueno and his research team, including colleagues at the UCLA School of Dentistry (USA), cultured osteoblasts on UV-treated titanium surfaces. On these surfaces, cells produced fewer ROS and pro-inflammatory cytokines, and the level of DNA damage was reduced. Furthermore, the antioxidative capacity was not depleted and ROS production was reduced, even in the presence of an oxidative stress inducer. The findings suggest that UV treatment induces a novel antioxidant capability in titanium.

Biomaterials, doi:10.1016/j.biomaterials.2016.08.050

Intracellular ROS levels in osteoblasts attached to titanium surfaces



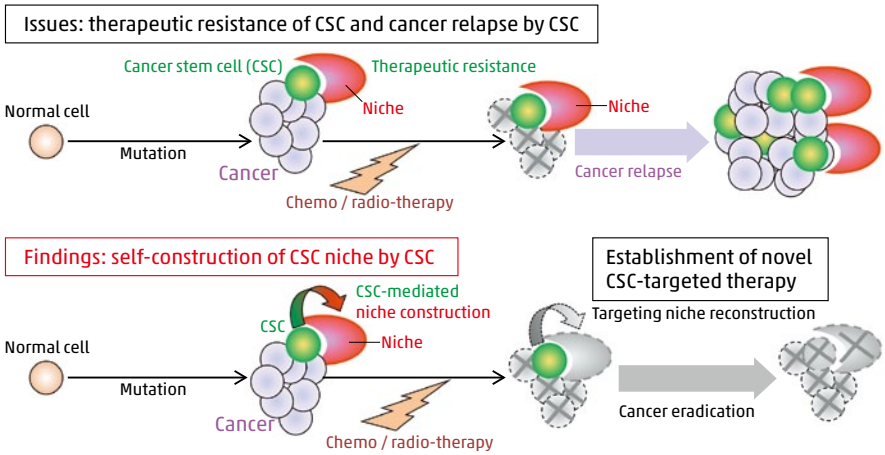
Representative dual-stained images for ROS (green) and cyto-skeleton (red). The number of cells attached to UV-treated surfaces was significantly higher than to untreated surfaces. The cells were obviously larger on UV-treated surfaces than on untreated surfaces, and the ROS signal was substantially decreased by UV-treatment.

Synthetic mimic reveals glioma cancer stem cells organize their own microenvironment

Cancer stem cells (CSCs) are an obvious target of tumor therapy, but their resistance to chemotherapy and radiotherapy has focused attention on disrupting the niche microenvironment that maintains them. TMDU-led research by Professor Tetsuya Taga and international colleagues used a synthetic polymer-based scaffold to create a mimic of the niche for glioma CSCs (GSCs), and identified extracellular matrix (ECM) and iron as key components of the GSC niche. The team showed that cultured GSCs were supported by a population of differentiated stem-cell progenies expressing ECM and transferrin (a protein involved in iron uptake). Similarly, in tumor xenografts, GSC-derived vascular endothelial cells were found to be a source of ECM and to behave as a drug barrier would against an experimentally administered anticancer drug. Further evidence that the GSCs organize their own niche was the observation that GSCs enable recruited monocytes to differentiate into protumoral macrophages that store iron. These findings reveal the adaptive capacity of GSCs, which could further the development of cancer therapies.

Stem Cells, doi: 10.1002/stem.2299

The concept of cancer eradication by targeting CSC-mediated niche construction



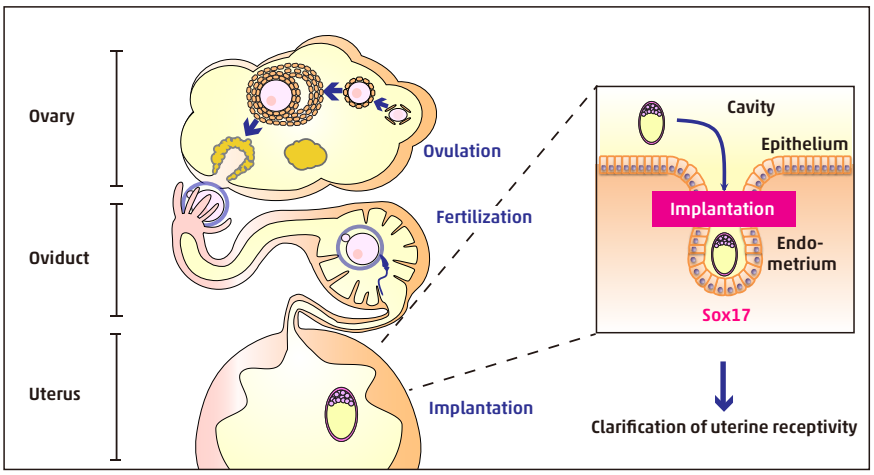
CSCs, a key driver in cancer progression, therapeutic resistance, and relapse are often proposed as a promising target for cancer eradication. As CSCs are maintained by the surrounding microenvironment, called a niche, disrupting the CSC niche is theoretically reasonable as a way to impair the CSC pool and thereby inhibit cancer progression and relapse (upper panel). Notably, we found that CSCs construct their own niche, and thereby adapt at sites where the niche is missing or disrupted. This discovery will hopefully open a new therapeutic path for cancer eradication (lower panel).

A novel role for Sox17 in embryo implantation

Embryonic implantation occurs only when the uterine endometrium is in a receptive state. Ovarian hormones orchestrate multiple signaling events to achieve implantation and establish pregnancy. During implantation, the expression of *Sry*-related high mobility group box gene-17 (*Sox17*) is elevated at the embryo attachment site. However, the role of Sox17 in implantation is unknown.

In a new study, Professor Masami Kanai-Azuma and colleagues at TMDU and other institutions examined fertility in mice with a heterozygous loss of *Sox17*. Mutant females produced fewer offspring, but ovulation, fertilization, blastocyst formation, and oviduct and uterus morphology were normal. Examination of the uterus at the time of implantation revealed fewer implantation sites in mutant females. Implantation also failed when wild-type embryos were transferred to the uterus of mutant females, excluding the possibility that problems with the embryos were responsible. The findings reveal a novel role for Sox17 in uterine receptivity to embryo implantation.

Uterine *Sox17* expression is necessary for embryo implantation



Sci. Rep., doi: 10.1038/srep24171

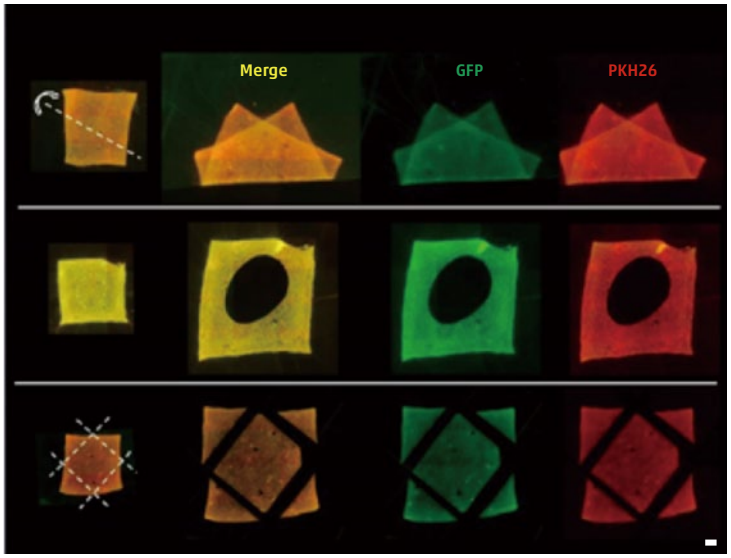
Photolithography-based cell transfer technology improves bone regeneration

Obstacles have remained for the treatment of bone defects using tissue engineering approaches. In this study, researchers developed a new technique, called cell transfer technology, whereby two types of cells can be attached to a scaffold in patterns that can be precisely controlled. Specifically, osteoblasts and stem cells from tooth ligament were attached via photolithography onto the amnion as a scaffold in overlapping layers in which each layer exclusively contained one type of cell.

Junior Associate Professor Kengo Iwasaki of TMDU led the team in transplanting this engineered structure onto mouse calvarial bone defects. As a result, more rapid bone formation and healing were observed, owing to the transplant closely mimicking native features. The use of the amnion-based scaffold also maintained the integrity of the structure despite folding and cutting, enabling modification of transplants to fit particular defects.

Sci. Rep., doi: 10.1038/srep33286

Stability of transferred cells upon trimming and deformation of amnion



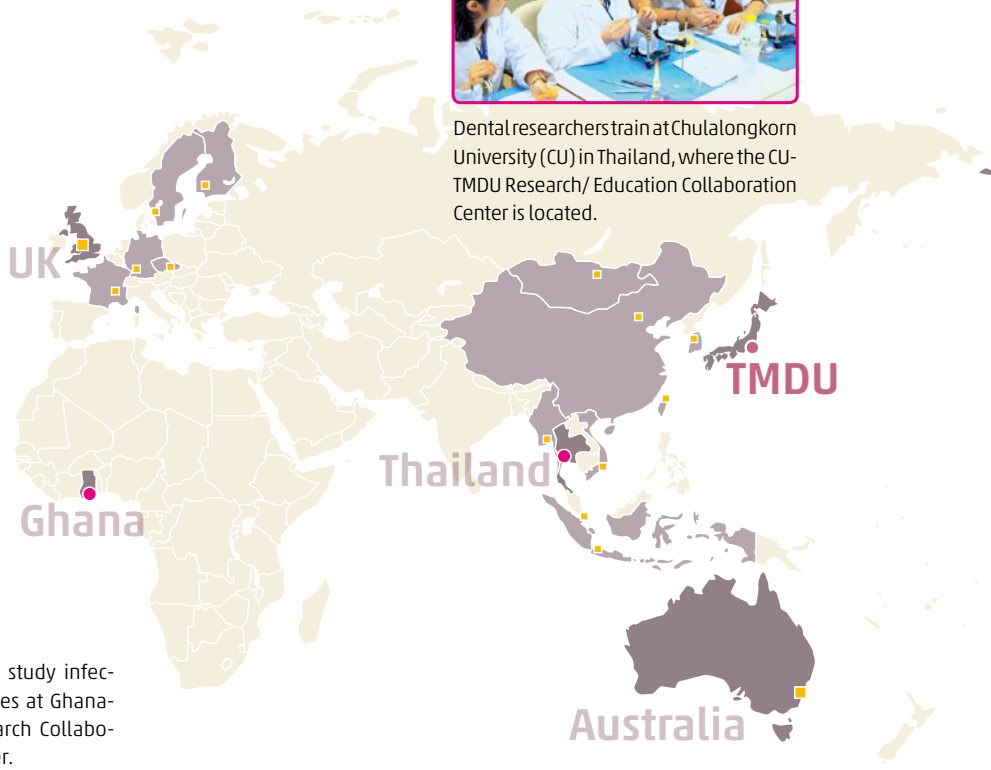
Fluorescence microscopic images of amnion holding double-layered cells after deformation (top), hole-cutting (middle) and trimming (bottom) of the membrane. After double-layered cell transfer, the amnion was folded along the dotted line (top). A circular hole was cut in the center of the cell-transferred amnion (middle). Four corners were trimmed along the dotted lines after double-layered cell transfer (bottom). Despite the deformations and trimming of the cell transferred amnion, the cells stably adhered onto the scaffold material. Green (GFP): First layer cells. Red (PKH26): Second layer cells. Bar = 1 mm

Sharing expertise and groundbreaking research around the world

Our international exchange activities in research and education are based in three centers, in Ghana, Thailand and Chile. We further promote educational collaboration with Harvard Medical School, Imperial College London and Australian National University. We also have 98 affiliated schools in 38 countries.

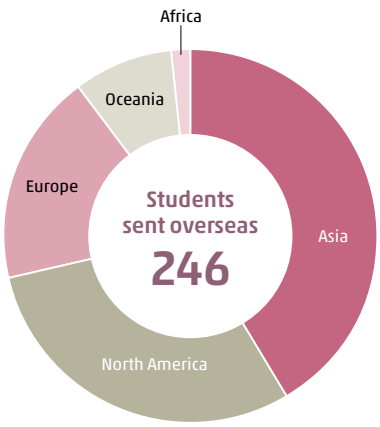
Dental researchers train at Chulalongkorn University (CU) in Thailand, where the CU-TMDU Research/ Education Collaboration Center is located.

Students join in an exchange program at Imperial College London, UK.



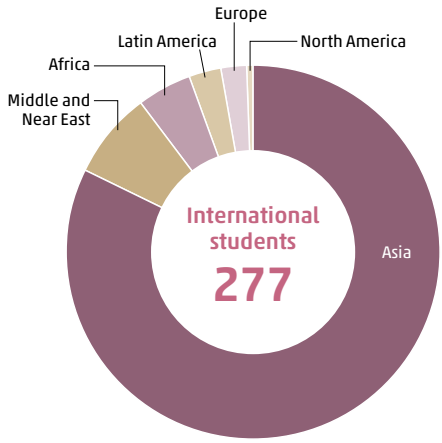
Researchers study infectious diseases at Ghana-TMDU Research Collaboration Center.

- Our international collaboration centers
- Our representative international education partners



About 11% of 1,200 eligible undergraduate students and about 8% of TMDU's 1,492 postgraduate students study abroad.

March, 2016



International students make up about 15% of TMDU's postgraduate students.

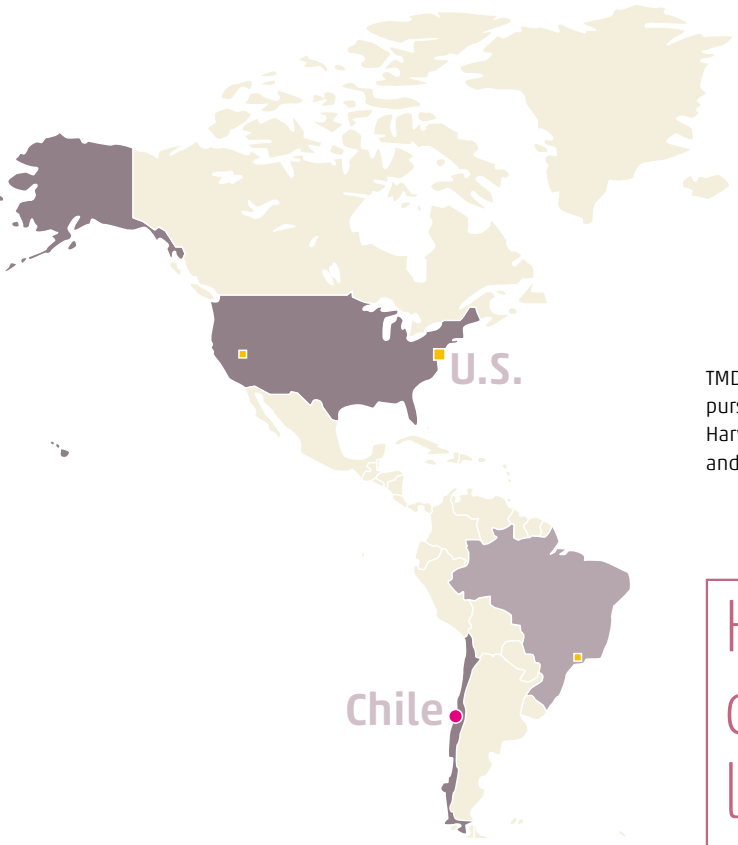
May, 2016

International students participate in exchange program with Australian National University.

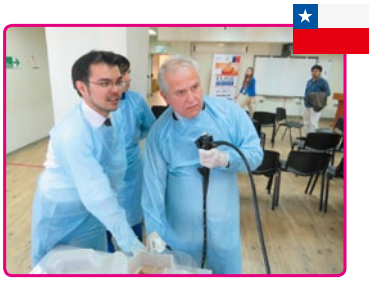
International researchers prepare traditional Japanese sushi-roll, "Eho-maki."

Students visit local park for viewing of the cherry blossoms, "o-hanami."

International students take Japanese calligraphy lesson.



TMDU's medical students pursue clinical training at Harvard Medical School and University of Nevada.



In Chile, at TMDU's Latin American Collaborative Research Center, doctors from TMDU and Clinica Las Condes work on a project to prevent neoplasia of the colon and rectum.

How do you like life at TMDU?

To be at TMDU is a privilege. Not only is it an opportunity academically, as I have had the opportunity to learn from the best researchers and professors, but also an opportunity where I have felt socially integrated by the community. If I could choose again, I would not hesitate: TMDU.

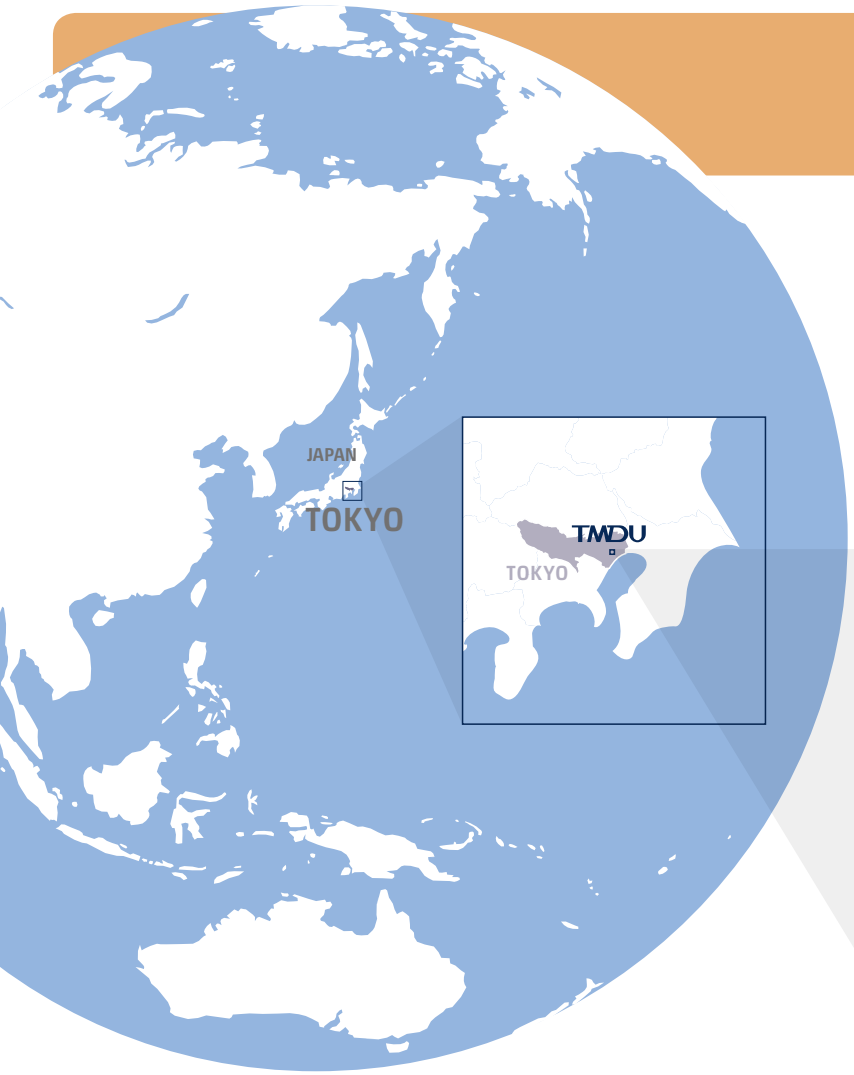
Jorge Espigares
(Spain)

Manila Nisha Gowri
(India)

I felt everything is set up to get the most from our studies! We have access to cutting-edge technology and world-class facilities at TMDU. I have gained good opportunities to present my research at occasions like academic meetings, symposiums and international conferences. In addition, TMDU has become more global in every aspect. Life as an international student is pleasant, thanks to the staff, who are always there for us.

TMDU's community is superb -- the medical presentations, lectures, patients cases, research papers and overall background of the professors. It makes me realize that I'm in the right place. Sometimes it's easy to lose oneself in all the projects, academic and extra-curricular commitments. This engagement with the university makes you fall in love with TMDU.

Andres Mora, MD
(Ecuador)



Main campus of TMDU (Ochanomizu / Yushima District)

TMDU Research Activities 2017

Cultivating professionals with knowledge and humanity, thereby contributing to people's well-being

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