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Keynote Lecture | KL | The Japanese Society for Microcirculation: Past, Present & Future

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On August 10, 1971, Dr. Masaharu Tsuchiya established "Gathering of Microcirculation Researchers" in consultation with founders, Dr. Takehiko Azuma, Dr. Makishige Asano, Dr. Yoshio Mishima, and Dr. Ryu Nakayama. This workshop was a forum for exchanging opinions on the microcirculatory system in various parts of the body, whether basic or clinical. Held from the first "Gathering of Microcirculation Researchers" (February 14, 1976) to 26th, during that time, Tokyo International Symposium on Microcirculation (July 26, 1981, at Sasagawa Hall), and Symposium on INTRAVITAL OBSERVATION OF ORGAN MICROCIRCULATION (June 18, 1983, at Tokai Alumni Association) were held. Before that, in January 1977, Prof. Tsuchiya was appointed as a professor at Keio University School of Medicine, and was quickly recognized in the fields of microcirculation to establish a study group and further developed into an academic society. This study group developed into the "Japanese Society for Microcirculation" on February 16, 1985. Then, Prof. Tsuchiya, as a President, held the 4th World Congress for Microcirculation (1987. 7. 26- 8. 2, Keio Plaza Hotel, Shinjuku-ku, Tokyo) in Tokyo with 440 foreign delegates from 27 countries and 500 domestic delegates. Without Prof. Tsuchiya, we cannot talk about the Japanese Society for Microcirculation today. After Prof. Tsuchiya passed away, Prof. Hiromasa Ishii was appointed as the second President in 2001, and then Prof. Makoto Suematsu was appointed as the third President in 2008 and held the 10th World Microcirculation Society in Kyoto (Kyoto International Conference Center, Kyoto) with 240 delegates from overseas (30 countries) and 164 delegates from Japan. On March 26, 2016, I was appointed as the fourth president of the Japanese Society for Microcirculation.

Now, life science research in the coming era is an area that covers a broad range from basic to clinical, such as microcirculatory science. For this purpose, the activities of this academic society are extremely important, encompassing many interdisciplinary areas such as basic medicine, clinical medicine, engineering, pharmacy, agriculture, information science, and sociology, and between researchers in Japan as well as abroad. It is necessary to foster the next generation while promoting exchanges and joint research. It is also important to carry on new academic challenges positively and promptly while inheriting the brilliant flow established by seniors and respecting tradition. In this lecture, I want to share this spirit with you again.

Symposium 1

Microcirculation and Metabolism
Brainstorming technologies and ideas

SY1-3 | Alteration of gut microbiota could be a novel therapeutic strategy for preventing cardiovascular diseases

Tomoya Yamashita
Kobe University Hospital

Basic and clinical evidence has revealed the critical roles of gut microbiota in host physiology including immune responses and metabolism. The gut microbiota and their related metabolites were shown to be associated with many diseases including cardiovascular diseases (CVD) and thought to be therapeutic targets. For example, high plasma concentration of trimethylamine *N*-oxide (TMAO), one of gut microbiota-associated metabolites, was shown to be associated with the incidence of cardiovascular events and the prognosis of heart failure.

We investigated the gut microbiota of coronary artery disease (CAD) patients and found that specific bacteria, *Bacteroides vulgatus* and *dorei* (*2 species*), were decreased in CAD patients compared with controls. Because the predominance of the phylum Bacteroidetes was

shown to be related to decreasing body weight, we hypothesized that *Bacteroides 2 species* could be athero-protective bacteria in our body. Oral administration of the cultured lived *Bacteroides 2 species* (5.0×10^9 cfu x5/week) to apolipoprotein E-deficient (apoE-/-) mice for 10 weeks significantly inhibited atherosclerotic lesion formation by decreasing inflammatory responses, including reduction in plasma cytokine and lipopolysaccharide (LPS) levels. Fecal LPS activities evaluated by the Limulus test were also significantly reduced probably due to altering gut bacterial contents in *Bacteroides*-treated mice compared with non-treated controls. Further, LPS derived from the *Bacteroides 2 species* was shown not to stimulate Toll-like receptor 4 (TLR4) strongly compared with that of *Escherichia coli*. Now we are trying to clarify the detailed mechanisms of preventing atherosclerosis by the anti-inflammatory *Bacteroides 2 species* and to develop gut bacterial drugs.

Further, we examined and clarified the roles of gut microbiota and their metabolites (e.g. TMAO) in the pathophysiology of heart failure. We would like to review the role of gut microbiota in atherogenesis and present the progress of our basic and translational research implying the possible novel therapeutic strategies of altering gut microbiota and their functions for preventing CVD.

SY1-4 | Porous materials for controlling bioactive gases

Shuhei Furukawa
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Gaseous molecules such as nitric oxide (NO) and carbon monoxide (CO) are recently known to be biological signaling molecules (gasotransmitters) working both for intercellular communications and intracellular regulations. In particular, NO is one of the most investigated gasotransmitters, having important roles in numerous signaling events as well as therapeutic potentials. However, the design of functional scaffolds or devices that can release NO with precisely controlled timing, dosage and location remains challenging, due to handling issues that arise from their high reactivity and physical state. Here, we show a synthetic strategy for developing spatiotemporally controllable NO-releasing platforms based on photoactive metal-organic frameworks (MOFs). By organizing molecules with poor reactivity into framework structures of MOFs, we observe increased photoreactivity and adjustable release using light irradiation. We further embed photoactive MOF crystals (NOF-1 = nitric oxide framework-1) in a biocompatible matrix, PDMS, leading to a functional cell culture NOF-1/PDMS substrate, and demonstrate precisely controlled NO delivery at the cellular level via localized two-photon laser activation. The biological relevance of the exogenous NO produced by this strategy is evidenced by an intracellular change in calcium concentration, mediated by NO-responsive plasma membrane channel proteins. We further shape photoactive MOF crystals at the mesoscale by coordination modulation and confirm its delivery inside cell and NO stimulation at the subcellular resolution.

Special Lecture 2 | SL2 | Imaging metabolomics for dissecting cancer metabolism

Makoto Suematsu
Professor, Department of Biochemistry, Keio University School of Medicine

High-throughput immunofluorescence proteomics screening system for clinical pathology using tissue microarray identified overexpression of cystathionine gamma-lyase (CSE) as an independent biomarker distinguishing non-responders from responders of ovarian carcinoma to platinum-based chemotherapy after debulking surgery. Among varied classification of pathological types, clear cell carcinoma (CCC) constituted a major histological type with significantly greater CSE expression than others, we aimed to detect multiple thiol metabolites derived from CSE at once, but high-energy laser application used in conventional imaging mass spectrometry hampers accurate detection of reductive metabolites responsible for CCC chemoresistance. We thus examined roles of CSE-derived metabolites for the chemoresistance by applying large-area surface-enhanced Raman spectroscopy (L-SERS) (Shiota M, et al. Nat. Commun. 2018) to compare sulfur-containing metabolites between CCC tissues and those from serous carcinoma (SC) as controls. The L-SERS substrates enabled us to semi-quantitatively visualize SERS signals derived from vacuum-dried tissue slices without any staining or labeling, and to detect glutathione, polysulfides and hypotaurine on tissues. This method allowed us to visualize polysulfides (PS), but neither glutathione nor hypotaurine in CCC, and the PS-signal was significantly greater in CCC than in SC. Thus, on-tissue PS detection by L-SERS is useful to predict platinum-based chemosensitivity. Combination of imaging MS with SERS technology allows us to assess heterogeneous metabolism among cancer cell nests and the surrounding cancer stroma in the same tissue slices, benefiting a quality of clinical-pathological diagnosis.

Educational Lecture (Morning Seminar) | EL | A role of cerebral small vessel and its pathological state

Toshiki Mizuno
Kyoto Prefectural University of Medicine

Cerebral small vessel consists of penetrating arteries, arterioles and capillaries. Diameter of these vessels are between 100 and 200 μm, and containing smooth muscle cells, endothelial cells and pericytes. Recently, a role of pericyte is attenuated because pericytes regulate capillary blood flow as well as the blood-brain barrier. Pericytes maintain homeostatic and hemostatic functions in the brain and sustain the blood-brain barrier. Also, pericytes work as the clearance and phagocytosis of cellular debris. This means pericytes are important for an emission system as well as a supply system.

If these systems are disturbed by disease, sequential event occur in the brain. In a case of hereditary cerebral small vessel disease,

the dilation and contraction of cerebral small vessel and capillaries are restricted due to abnormal accumulation of extracellular matrix, such as extradomain of NOTCH3, tissue inhibitor of metalloprotease3 (TIMP3) In CADASIL and fibronectin in CARASIL.

This abnormal accumulation of extracellular matrix induces white matter disease due to cerebral blood insufficiency or hypoxia. It also induces cerebral infarction due to hypoxia or obstruction of cerebral arteries. This abnormal accumulation of extracellular matrix also disturbs the vulnerability of cerebral wall and finally induces cerebral hemorrhage.

Recently, lymphatic drainage of Interstitial fluid (ISF) from brain parenchyma along basement membranes in walls of cerebral capillaries and arteries was proposed from the experiments using fluorescent tracers. This hypothesis is reasonable for explaining A-beta deposition along the cerebral arteries in Alzheimer's disease as cerebral amyloid angiopathy. Abnormal accumulation of extracellular matrix might disturb an emission system from the intraparenchymal cerebral tissue to the general circulation via cerebral vessel. This pathological process might be related to the degeneration of cerebral cortex and white matter, which induce finally dementia.

I talk about a role of cerebral capillaries and this disturbance cause not only vessel disease also dementia.

Special Lecture 3 | SL3 | Bone marrow microenvironmental niches for hematopoietic stem cells and hematopoiesis

Takashi Nagasawa
Graduate School of Frontier Biosciences, [2]Graduate School of Medicine, Osaka University

All blood cells are generated from hematopoietic stem cells (HSCs) in the bone marrow in the adult. HSCs are in contact with and maintained by restricted microenvironments, termed niches; however, the identity of HSC niches has been a subject of longstanding debate. We have focused our analysis on the chemokine CXCL12 and its primary receptor CXCR4, which are essential for homing and maintenance of HSCs and development of immune cells (1, 2) as well as blood vessel formation in heart and gastrointestinal tract (1). We identified a population of mesenchymal cells with long processes, expressing high amounts of CXCL12, termed CXCL12-abundant reticular (CAR) cells in murine bone marrow (2, 3). We revealed that most HSCs were in contact with CAR cells (2) and that ablation of CAR cells *in vivo* severely impaired the production of CXCL12 and stem cell factor (SCF), and led to a marked reduction in the numbers of HSCs and hematopoietic progenitors, including lymphoid, erythroid and myeloid progenitors (3). Furthermore, we found that the transcription factors Foxc1 and Ebf3 were preferentially expressed in CAR cells and essential for maintenance of HSCs and hematopoietic progenitors in the marrow and that Foxc1 and Ebf3 were essential for inhibiting differentiation of CAR cells into adipocytes and osteoblasts, respectively (4, 5). On the other hand, we revealed that CAR cells were self-renewing mesenchymal stem cells, which give rise to all osteoblasts and adipocytes in adult bone marrow, using lineage-tracing (5). Thus, the bone marrow-specific population of mesenchymal stem cells, CAR cells are the major cellular component of niches for HSCs and hematopoiesis. We will show the features of CXCL12-expressing cells in human bone marrow.

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Presidential Lecture | PL | Microbiome, Microcirculation and Medicine

Yuji Naito
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Recent research using a next-generation metagenome analysis has demonstrated that commensal microbiome regulates the maturation of the mucosal immune system, while the pathogenic microbiome causes immunity dysfunction, resulting in disease development. Intestinal epithelial cells and immune cells cross-talk as the first line of defense in the gut against microbiome, and are strongly influenced by the products, mainly short-chain fatty acids (SCFAs) and lactate, of commensal bacteria. The present talk will focus on the role of SCFAs, especially butyrate, in the maintenance of mucosal barrier function, microcirculation, and the circadian rhythms. During gut homeostasis, obligate anaerobic bacteria convert dietary fiber into fermentation products (butyrate) to maintain the epithelium in a metabolic state characterized by high oxygen consumption. This metabolic polarization of differentiated colonocytes maintains epithelial hypoxia (<1% oxygen) to limit the amount of oxygen diffusing into the gut lumen. Oxygen derived from microcirculation and butyrate from obligate anaerobic bacteria could support the mitochondrial function and ATP production. In addition, the blocking the diffusion of oxygen into the lumen could support the homeostasis of obligate anaerobic bacteria. Condition that shifts the metabolism of colonocytes, for example antibiotics treatment or inflammation, could cause an increase in the amount of oxygen emanating from the mucosal surface, thereby driving a shift in microbial community from obligate to facultative anaerobes, a hallmark of dysbiosis in the colon. Finally, we will discuss the differences in obligate and facultative anaerobes in older healthy subjects between Kyotango, one of the longevity areas, a provincial city located in the northern part of Kyoto Prefecture and Kyoto, an urban city located in the southern part of Kyoto Prefecture. Our data support that the alterations in the microbiota may provide new insights to consider the relationship between longevity and gut microbiota.

Memorial Lecture

Remembrance of Prof. Masaki Kitajima

ML | Expansion of Prof. Masaki Kitajima's achievements on the field of the microcirculation | —From silicon rubber cast to the developments of the fluorescence guided surgery—

Masashi Yoshida
Department of Surgery, International University of Health and Welfare Hospital

On May 21st, 2019, the world of surgery changed into one where Prof. Kitajima does not exist. His achievements cover a wide range of medicine and Japanese government needed him as an opinion leader of science. Even though his achievements were great and macroscopic, his life as a researcher started from microscopic observation of the microvasculature of intestinal anastomosis when he was a resident in Keio University Hospital. He continued observation of the gastric microvasculature using silicon rubber cast during his fellowship in department of surgery, Massachusetts General Hospital, Harvard Medical School. When he came back to Japan, he was appointed as a lecturer, and later, professor of Kyorin University School of Medicine. I was graduated from Kyorin University when he was associate professor and he had been supervisor of me for 31 years. In his Kyorin University days, he investigated many factors of microcirculatory disturbance including observation of the silicon rubber cast, blood flow assessment and prostaglandins. Then he moved to Keio University as a Professor and Chairman, Department of Surgery. He advocated 3 main themes of research, the liver transplantation, minimally invasive surgery including laparoscopic and robotic surgery, and surgical oncology. His idea of protecting microcirculatory disturbance mainly contributed to liver transplantation and Prof. Kitajima and I could continue to study on microcirculatory disturbance of the stomach in the laboratories of transplantation group and Department of Gastroenterology and Hepatology after I moved to Department of Surgery, Keio University following Prof. Kitajima. Especially, *in vivo* observation of gastric microcirculation was very exciting for us. We could see behavior of fluorescence-labeled leukocytes using fluorescence microscopy. We always thought about the possibility of intraoperative assessment of microcirculation of the gastrointestinal tract. After serving out his term as the professor and chairman in Keio University, he moved to International University of Health and Welfare (IUHW) as vice-president of the university and the director of IUHW Mita Hospital. (Later, he appointed as vice chief director of IUHW after he contributed to the establishment of Faculty of Medicine.) I also moved to IUHW Mita Hospital and, we could be involved in the development of the first brightfield, full-color fluorescence camera for surgery. Using this camera, observation of the hepatocellular carcinoma, cholangiography, blood flow of the gastrointestinal tract sentinel lymph nodes can be done simultaneously while operation. The fluorescence guided surgery became quite popular and Prof. Kitajima played an important role in the establishment of the Japanese Society for Fluorescence Guided Surgery. In the last animal experiments in the "Kitajima era," we hypothesized that fluorescence of fluorescein is suitable for the intraoperative assessment of microcirculation of gastrointestinal tract. We will continue to investigate the fluorescence of fluorescein.

Symposium 2

Microcirculation and Pathophysiology

SY2-1 | Therapeutic countermeasures to attenuate microcirculation disorders in skeletal muscle

Hidemi Fujino
Kobe University Graduate School of Health Sciences

Skeletal muscle capillaries run tortuously along muscle fibers. These capillaries are connected with anastomoses, which run orthogonally to muscle fiber direction like parallel rungs of ladder. In skeletal muscle, capillary is increased by the augmentation of muscle activity, exercise or electrical stimulation, and is decreased by disuse, denervation and metabolic disorders. We found that disuse resulted in a decrease in capillary volume and capillary luminal diameter. In addition, the number of anastomoses and the tortuosity of muscle capillary decreased and endothelial cell apoptosis was observed under disuse condition. In diabetes, skeletal muscle has also been associated with a reduction in capillary volume and diameter. Thus, disuse and diabetes alter capillary hemodynamics, induce capillary regression, called "ghost vessels," in skeletal muscle.

It is well known that exercise increases angiogenesis, and is effective in maintaining and improving oxidative metabolism in skeletal muscle. Exercise increased blood flow to skeletal muscles, which induced an increase in capillary. We found that low- and moderate-intensity exercise trainings appear to be a strong countermeasure for the capillary regression and reduction in the levels of angiogenic factors in the diabetic skeletal muscles. Thus, exercise resulted in an adaptive increase in capillary of impaired skeletal muscle.

Disuse-induced capillary regression is associated with increased oxidative stress. Previous studies have suggested that anti-oxidative nutrients can attenuate skeletal capillary regression induced by disuse. The administration of astaxanthin attenuated the increase in oxidative stress, and up-regulated the angiogenic factors in disused skeletal muscle. In addition, the VEGF-to-thrombospondin-1(TSP-1) ratio was higher in the astaxanthin treated muscle than in atrophied muscle. The results of this study demonstrate that astaxanthin is effective in preventing capillary regression in skeletal muscles exposed to chronic periods of decreased loading and activity levels. The administration of Brazilian propolis also prevented the overexpression

of oxidative stress due to a chronic decrease in neuromuscular activity and capillary regression by suppressing anti-angiogenic signaling and stimulating pro-angiogenic factors within the atrophied muscle. Furthermore, we reported that the administration of Enterococcus faecium strain R30 resulted in an increase in the red blood cell velocity in the capillaries of the skeletal muscle via muscle sympathetic nerve activity, and a decrease in capillary regression under disused condition. Therefore, the supplementation of these nutrients may be an effective countermeasure to the detrimental effects under disuse condition.

From a therapeutic viewpoint, these results suggest that exercise and nutrient administration may be a useful strategy to use during the rehabilitation period for conditions involving muscle dysfunction.

SY2-3 | Effect of pre and postconditioning on ischemic/reperfusion injury

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The blood reperfusion occurs in ischemic tissue causes tissue damage, because the production of various toxic substances is induced in the microcirculation (ischemic/reperfusion injury: I/R injury). Mechanisms such as disorders caused by the production of active radicals and various chemicals and disorders based on the interaction between activated neutrophils and vascular endothelial cells are considered.

It is well established that I/R injury is reduced by preconditioning with brief periods of vascular occlusion (ischemic preconditioning) or a variety of pharmacological agents. In addition, more short periods of ischemia followed by also more short periods of reperfusion immediately after the ischemia and just before the phase of reperfusion attenuates postischemic injury (ischemic postconditioning). Some of the components of signal transduction in preconditioning and postconditioning are the same, but the others are different. We have previously reported several kinds of the agent, such as ethanol, redox and platinum nanoparticles and some gas molecules, could make the preconditioning state against I/R injury in the murine small intestine. We would like to show our recent data and review the current state of preconditioning and postconditioning study.

Free Paper | F-01 | Optogenetic manipulation of cerebral microcirculation by transcranial photostimulation to the channelrhodopsin-2 expressing mouse brains

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Objectives: The present study aimed to characterize spatiotemporal dynamic changes in cerebral microcirculation during transcranial photostimulation to the anesthetized mouse brains that expressed a light sensitive cation channel (channelrhodopsin-2; ChR2) in the vascular smooth muscle cells.

Methods: Transgenic mice (16-28 g, $N = 9$) that expressed ChR2 in the vascular smooth muscle cells were used for the experiments under the experimental protocols approved by the institutional animal care and use committee. Spatiotemporal dynamic changes in cortical blood flow were measured with laser speckle flowgraphy, while focal photostimulation (0.5 mm in diameter) was transcranially delivered to the cortex of the urethane-anesthetized animals. Physiologic conditions of the animals (body temperature, blood pressure, heart rates, and breathing rates) were monitored throughout the experiments.

Results: Transcranial photostimulation evoked transient decreases in cortical blood flow in a stimulation power-dependent manner. The responses spread from the irradiated spot to broad cortical regions. In separate experiments, direct visualization of the cerebrovascular responses with *in vivo* two-photon microscopy revealed that only arterial vessels transiently constricted (10%-20% relative to the baseline diameters) following the photostimulation, while venous vessels remained unchanged. Furthermore, spot excitation to a single cross-section of the ChR2-expressing arteries (both pial and penetrating arteries) evoked a fast spread of the vasoconstrictive responses along the arterial vessels, but not venous sides. Physiologic conditions of the animals were observed to be stable during photostimulation under our experimental conditions.

Conclusion: Transcranial photostimulation evoked wide-spread transient vasoconstriction in the cerebral arteries and thus regional hypoperfusion in the transgenic mice expressing ChR2 in the vascular smooth muscle cells.

F-02 | Dynamic response of arterial diameter and red blood cell velocity in capillaries associated with cortical spreading depolarization/depression

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Background: Control of red blood cell (RBC) velocity in capillaries is essential to meet local neuronal metabolic requirements, although changes of capillary diameter are limited. Cortical spreading depolarization/depression (CSD), which is thought to be involved in various neurological disorders, is accompanied with mass depolarization of neurons and glia and marked changes of blood flow. OBJECTIVES: To further understand the microcirculatory response during CSD, we investigated changes of RBC velocity in intraparenchymal capillaries and the concomitant changes of arterial diameter.

Methods: In urethane-anesthetized Tie2-GFP mice (N = 21), sequential images were acquired for 60 sec with a high-speed camera (125 fps) laser-scanning confocal fluorescence microscope around the time of CSD induction, with simultaneous recording of DC potential and measurement of regional cerebral blood flow (rCBF) by laser Doppler flowmetry. The velocity of fluorescence-labeled RBCs in layer I was analyzed with KEIO-IS2 software using MATLAB and the diameter of pial/terminal arteries was analyzed with ImagePro.

Results: In total, 265 capillaries were successfully evaluated in 18 mice (5-19 capillaries/mouse). Basal RBC velocity and arterial diameter were 0.91 ± 0.36 mm/s and 24 ± 5 μm, respectively. RBC velocity was significantly increased (p < 0.01) prior to arterial constriction/dilation concomitantly with repeated occurrence of CSD. During the first CSD, the RBC velocity markedly decreased in parallel with marked arterial constriction. The velocity then returned to around the basal level, while post-CSD oligemia with slight vasoconstriction remained.

Conclusion: Taken together with our previous findings, this study supports the idea that RBC flow changes are not entirely dependent upon arterial regulation, and neuro-capillary coupling may play a role in meeting neural metabolic demand.

This study was performed with the approval (No.09058) of the Animal Ethics Committee of Keio University (Tokyo, Japan).

F-03 | Flow velocity mapping of the human nailfold microcirculation imaged with capillary flow video microscopy

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Objectives: An image-based velocimetry method was previously developed for fluorescent video microscopy images without use of the high-speed camera. In the present study, the method was applied to the video microscopy images of the human nailfold microcirculation to test whether the flow velocity can be measured based on native contrast differences among the flowing blood cells.

Methods: White-LED irradiated reflection images of the nailfold microcirculation in the healthy subjects, captured with capillary flow video microscopy (Toku Corporation), were used for the image analysis. In this image, red blood cells in the vessels showed a dark color due to absorption of the light by hemoglobin, whereas other blood cells and plasma showed a brighter color because of non-absorption characteristics. The images were analyzed with Matlab-R2019b. The vessel region was first extracted through the U-Net (a conventional neural network), and further divided into straight and curve segments. A kymograph was then created along the centerline of the vessel segments over measured time. The image represents a stripe pattern which is caused by motion of the contrast between the red blood cells and others in an axial direction of the vessel. Because an angle of the stripe represents flow velocity in principle, the major angular component was determined using Radon transform image. The obtained velocities were finally compared with the manually-measured flow velocities of the blood cells.

Results: The measured velocities with the present method (0.1-0.4 mm/s, n = 5) were in good agreement with the manually-measured one. A good linear correlation (R = 0.92, p = 0.03) was observed for those two measurements.

Conclusions: The proposed method with a kymograph along the vessel centerlines successfully determines the spatiotemporal changes in microvascular flow velocity imaged with capillary flow video microscopy.

F-04 | Effects of life style differences on the human nailfold microcirculation

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Objectives: Capillary shape and blood flow are believed to sensitively reflect healthy conditions of the systemic circulation. Video microscopy of human nailfold microcirculation has revealed subject-dependent variations in the morphological features of the capillaries. The present study was aimed to determine confounding factors that influence on the vascular structures and/or flow parameters in the human nailfold microcirculation.
Methods: A total of 42 healthy subjects were agreed with the measurements of their nailfold microcirculation. The protocols were approved by the institutional ethics review committee. The nailfold microcirculation of the subject's ring finger of the non-dominant hand side was measured with capillary flow video microscopy (Toku Corporation) at a rate of 30 frame per sec with 700 by 525 μm (0.7 μm/pixel). All images were analyzed with Matlab-R2019b. Displacement of the images in a radial direction to the vessel orientation was corrected, and the images were averaged over 10-sec measured periods. The vessel region on the images was extracted, and the vessel structures were quantified by measuring a segment length, diameter, and a radius of the curve approximated. In addition, flow velocity and amplitude spectrum of the pixel intensity fluctuations were characterized. All measured parameters were then compared with the subject's age and daily activities (smoking, drinking, and exercise) asked by the questionnaire form, by using discriminant analysis methods.
Results: We observed that the vessel diameter (a mode value of the vessel segments) significantly differed between the young (less than 30 years; 12 ± 3 μm, N = 28) and middle-aged groups (over 30 years; 15 ± 4 μm, N = 14). Also, statistically significant differences were observed for the high-frequency oscillation amplitudes of the pixel intensity between the exercised and non-exercised groups.
Conclusions: The significantly larger vessel diameters were observed in the aged subjects relative to the young subjects.

F-05 | Effect of the infrared rays given to the neck on the capillary blood flow rate of the finger: video motion analysis

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Objective: We examined the effect of infrared rays (IRR) from inorganic germanium (IGe) and those from LED given to the neck on the capillary blood flow rate (BFR) of the finger. IGe showed anti-working-stress effects on germanium-transistor radio assemblers in 1950s. Fujiyasu, professor of mechanical electronics, Shizuoka University, suggested that the radiation of IRR from IGe may be a cause of such biological effects. This has remained to be proven.
Methods: After the approval of the institutional ethical committee of Aoki Hospital for neuro-plastic surgery, we divided 30 subjects into 2 groups. With a video-microscope (TOKU capillaro®), the capillary blood flow of the left 4th finger was recorded and 2 capillaries were analyzed in each subject. We started with control recordings for 1 minute before treatments. Then as treatments, IGe group had an IGe necklace (metal alloy composed of IGe, platinum and stainless steel, KHG®) put on for the 5-min recording. LED group received IRR radiation from LED lamp (SuperLizer PX®), beamed against the left subclavian artery at the middle of the clavicle, at a power of 50 w for the 2-min recording. Japanese Capillary Research Conference® analyzed the data. The sample size for each group was 8. We expressed data as mean(SD), and used paired t-test to compare BFR of before treatment with the after.
Results: The age distribution; IGe: 61 ± 12, vs LED 53 ± 17 (years). The treatments increased BFR by 16% in both groups, i.e. BFR before and after the treatments; IGe: 907 ± 193 and 1056 ± 233 ($p < 0.001$), LED: 800 ± 275 and 926 ± 353 ($p < 0.005$) (μm/sec).
Conclusions: IGe and IRR from LED given to the neck increased the capillary BFR of the finger in similar fashion. The biological effects of IGe may be caused by IRR from it.

F-06 | Age-related changes in the facial skin blood flow in healthy women examined by laser speckle flowgraphy

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Objective: We evaluated parameters for detecting age-related changes in the facial skin blood flow (FSBF) using laser speckle flowgraphy (LSFG).
Methods: The subjects were 183 healthy women (mean age: 46.7 ± 15.1 years) in their 20s and 70s, divided into two groups: 102 in

the young group (YG) (35.4 ± 8.9 from 20s to 40s) and 81 in the elderly group (EG) (61.0 ± 6.3 from 50s to 70s). After acclimating the subjects to a temperature and humidity environment of 24.0°C and 50.0% RH, resting FSBF was measured by LSFG. A morphing technique was used to define the left cheek among individuals. The blood flow waveform indices were created from the FSBF normalized by one heartbeat time as follows: rising slope: blood inflow into skin, BOT (blowout time): sustained blood flow, falling slope: blood outflow, fluctuation: fluctuation of blood flow, RI: peripheral vascular resistance (PVR).

Results: A significant negative correlation was found between age and the following parameters: the rising slope, BOT, and the falling slope. On the other hand, a significant positive correlation was noted between age and the following parameters: the fluctuation and RI. Combining this analysis for FSBF suggested that the ability to maintain the blood flow decreases with age. In the EG, rising slope, BOT, and falling slope were significantly lower, fluctuation and RI were significantly higher, the PVR increased with age, and blood flow fluctuated easily and decreased more rapidly after peaking.

Conclusions: LSFG using the evaluated parameters was used to compare individual FSBF without external stimuli. Moreover, as LSFG enables objective analysis of age-related changes in FSBF, it can be further applied to the study of blood flow changes in the medical and healthcare fields.

All experiments described in this study were approved by the Kao Corporation Ethics Committee (#2011-0858).

F-07 | Spatiotemporal correlation analysis of the capillary red blood cell flow and vessel diameters in the anesthetized rat cortex

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Background: Slow fluctuations of brain hemodynamics are thought to be driven by a change in activity of neurons and/or glial cells in the brain. To identify the source of brain hemodynamic fluctuations in relation to local brain activity, we developed an experimental method to fluorescently visualize a motion of the red blood cells (RBCs) with cellular activities simultaneously under two-photon microscopy. The purpose of the present study is to characterize the spatiotemporal fluctuations of the RBC flow in single capillaries and their correlations to the connecting capillaries in the anesthetized rat cortex.

Methods: Two transgenic rats that express fluorescent proteins in the erythrocyte membranes were used for the experiments. The experimental protocol was approved by the Animal Experiment Ethics Committee of the University of Electro Communications. A portion of the skull was replaced to a glass for transparency under isoflurane anesthesia. The RBCs flowing in the cortical microvessels were visualized with two-photon microscopy concurrently with fluorescently labeled blood plasma. For a capillary region, a number of pixels occupied with RBC or plasma were counted in each frame, and a ratio of the RBC to plasma area was determined in each vessel segment. Also, a number of the RBC stayed over consecutive 100 frames were counted to determine an apparent dwell time of the RBC in each pixel. Then, the maximum dwell time over single capillary segments was evaluated in each frame and compared with those of the other vessels, as well as a mean diameter of the measured vessels.

Results: We observed that the capillary RBC flow largely fluctuates over 60-sec recording time, whereas the capillary diameter stayed relatively constant, indicating that the RBC flow in the single capillary is independently altered without changes in the capillary diameters. Next, we compared the RBC fluctuations among the connecting capillaries, which showed that the correlation varied over the measured periods. The findings showed that the single capillary RBC flow is largely dependent on the fluctuations of the connecting capillary RBC flow, which could be regulated by pressure balances in the capillary networks.

F-08 | Intercellular transport of amyloid β *in vivo* observed with multiphoton microscopy

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Background: Amyloid β (Aβ) is one of the causative proteins found in Alzheimer's Disease (AD), and transcytotic delivery, perivascular drainage, and enzymatic/glial degradation are proposed as possible removal mechanisms. Here, we report the differences in the transport pathway between dextran and A applied on the brain surface.

Methods: Under isoflurane anesthesia, the skin covering left parietal skull of male mice was incised and a cranial window (3.5 mm in diameter) was opened with a dental drill. After removing the dura mater, HiLyte488-Amyloidβ1-40 (Anaspec), HiLyte488-Amyloidβ1-42 (Anaspec) or FITC-Dextran(MW 4.4 kDa or 40 kDa Sigma-Aldrich) was topically applied on the cortical surface and the window was closed with a cover glass. Tie2-GFP mouse (Stock Tg[Tie2-GFP]287Sato/J) was utilized to visualize the vascular endothelial cells. Three-dimensional imaging of the parietal cortex down to 300 μm was repeatedly conducted with multi-photon microscope (A1RMP+1080, Nikon).

Results: The high molecular-weight dextran distributed along the perivascular space of the penetrating arteries and veins, whereas small molecular dextran diffused through the brain parenchyma with much less distribution in the perivascular space. Aβdeposition was first observed around the arteries and then around the veins and in astrocytic endfeet. Finally it spread to the astrocytic somata throughout parenchyma. Aβaccumulation reached plateau in 40 minutes and was washed out in 80 minutes.

Conclusion: From our study, as one of the transport pathway of Aβ, cell-to-cell transport through astrocytic gap junctions (glial network) is suspected. Disturbance of the clearance system may contribute to the pathogenesis of AD.

F-09 | Morphologic classifications and locations of microaneurysms and clinical relevance in branch retinal vein occlusion

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Purpose: To classify microaneurysms (MAs) and investigate the relationships with retinal edema in eyes with branch retinal vein occlusion (BRVO).
Methods: Eyes with MAs due to BRVO that underwent optical coherence tomography angiography (OCTA) were enrolled. MAs on OCTA images were morphologically classified into six types: focal bulge, saccular, fusiform, mixed (saccular/fusiform), pedunculated, and irregular. The frequency, size, location, and relationships with retinal edema also were investigated. The Institutional Review Board of Nagoya City University Graduate School of Medical Sciences also approved this observational study (No. 60180149). The clinical trial was registered in University Hospital Medical Information Network Clinical Trials Registry (UMIN-ID: UMIN000033595).
Results: Twenty-four eyes of 23 patients (12 men, 11 women; mean age, 68.0 years) were enrolled. A total of 244 MAs were detected on the OCTA images. The focal bulge and saccular types accounted for over 70% of all MAs. Smaller MAs such as the focal bulge or saccular type also were detected both at the edge of the nonperfused areas (NPAs) and in collateral vessels. In contrast, larger MAs such as the pedunculated or irregular types tended to form at the edges of the NPAs. Older age, the presence of MAs in the collateral vessels, and the absence of pedunculated type were independent predictive factors for retinal edema but not the MA size, or presence in the retinal deep capillary plexus. After treatment, the mean retinal thickness decreased significantly, but the mean MA size remained unchanged.
Conclusions: OCTA enables morphologic classification, three-dimensional analysis, and investigation of the longitudinal changes of MAs with noninvasive volumetric quantification, leading to a better understanding of the pathology of MAs in eyes with BRVO.

F-10 | Immunohistochemical observation of the microvasculature of the human mammary tissue

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Objective: To elucidate the structure of microvasculature including blood and lymph vascular components of the human mammary tissue.
Materials and Methods: The material of the study is surgically resected breast tissues. They were made into FFPS and subjected to immunohistochemical reaction using antibodies of D2-40, anti-von Willebrand factor, anti-CD34, and anti-CD31.
Results: The lymphatic capillaries distributed in the outer peripheral zone of the mammary gland. In the mammary gland the lymphatic capillaries distributed around major and interlobular mammary ducts. In addition, between the major mammary ducts, a rich distribution of lymphatic capillaries in connective tissues was present. There was lymphatic capillary distribution in perilobular region but there was no intralobular distribution. The blood vessels distributed in the outer peripheral zone of the mammary gland sometimes in conjunction with lymphatic capillaries. In the mammary gland, the blood capillaries distributed around major and interlobular mammary ducts. There was blood capillary distribution in perilobular region and in intralobular region around interlobular mammary ducts.
Conclusions: Both blood vessels and lymphatic capillary distributed in periphery of mammary gland, around major and interlobular ducts, and around mammary lobules. But, in the mammary lobules only blood capillaries are present, and no lymphatic capillary distribution was demonstrated.

F-11 | Translocation Route of bacteria detected in liver and lung in the *Helicobacter suis*-infected mouse

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We have recently reported the formation of hepatic and pulmonary MALT lymphoma in the *Helicobacter suis (H.suis)*-infected mice, while the transmission mechanism from the stomach to these organs remains to be clarified. Thus, the present study was undertaken to elucidate the localization of *H. suis* and the microvascular network in the infected mouse.
Materials and Methods: We used a *H. suis* sample isolated from the stomach of a cynomolgus monkey and maintained in C57BL/6 mouse stomachs. Mucosal homogenates were used to inoculate C57BL/6 mice, which were then examined over 24 months. Macroscopic observations were carried out, and *in situ* hybridization method of the *Helicobacter species* by the microprobe method as well

as immunoistochemical analysis using the our newly established antibody against *H. suis* vac A paralogue, HsvA, and CD34, MadCAM-1 and podoplanin antibodies was performed at intervals over the observation period. This study was approved by the ethical committee of Kitasato Institute Hospital.

Results: The microcirculatory components distributed richly in the hepatic and pulmonary MALT lymphoma as well as in the stomach lymphoma. The immunoreactivity of *H. suis* was recognized within these microcirculatory units as well as in the surrounding tissues, and the lymphocytes were sometimes seen within the microcirculatory components. In conclusion, the transmission of *H. suis* through the microcirculatory system was shown to take part in the formation of liver and lung MALT lymphoma in the *H. suis*-infected mouse.

Applicants' Presentation for Young Investigator Award | Y-01 | Platelet-rich fibrin application promotes vascularization and osteogenic differentiation in periodontal tissue

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Objectives: Several methods that facilitate periodontal tissue regeneration are available in clinical dentistry. Platelet-rich plasma contains many growth factors, and may be effective in promoting the regeneration of tissue, like skin and bone. The aim of this study was to investigate the morphological and physiological effects of platelet-rich fibrin (PRF) application on periodontal tissue regeneration.

Methods: Premolars were extracted from beagle dogs, and PRF was applied to the alveolar sockets. PRF was prepared by collecting 10 ml of venous blood from each animal in a collection tube before tooth extraction. Based on advanced PRF technique, samples were centrifuged at 200 *g* for 8 min and allowed to stand for 10 min to facilitate clot formation. Morphological examination was performed to assess new bone formation, and the bone formation ratio was evaluated 14 and 30 days postoperatively. Laser Doppler flowmetry was performed to determine blood flow at the sockets. All animal experiments were performed in accordance with the prescribed protocol, which was reviewed and approved by the Institutional Animal Ethics Committee of Kanagawa Dental University (Permit Number 16045).

Results: Histological examination performed 14 days postoperatively revealed newly formed bone filling the sockets up to their center in the PRF group, and thick regular bone trabeculae arranged within porous bone tissue were observed 30 days postoperatively. Compared with the control group, the PRF group showed higher expression of osteocalcin and osteopontin in the newly formed bone. The bone formation ratio was also higher in the PRF group. Furthermore, intergroup differences in gingival blood flow were observed at each time point during the study.

Conclusions: The present study highlights that compared with the body's inherent capacity to induce vascularization and osteogenesis, PRF application produces these effects more rapidly. Therefore, PRF may be useful in clinical medicine to promote osteogenesis.

Y-02 | Indigo Naturalis increases ILC3 flowing through mesenteric lymphatic vasculature from intestinal mucosa

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Introduction: Indigo Naturalis (IN) is a traditional herbal medicine for treating chronic diseases such as ulcerative colitis. Recently, IN has been known as ligand of Aryl hydrocarbon receptor (AhR). It is reported that AhR is expressed in a broad field of immune cells isolated from various tissues and innate lymphoid cells (ILCs), and related with secretion of IL-22 which promotes gut homeostasis. ILCs have important effects and regulatory functions in both innate and adaptive immune responses. ILCs include ILC1, ILC2, ILC3 and immature ILC. ILC3, the most abundant subset in the intestine, is reported to be rapid source of protective cytokines following initial exposure to a variety of pathogens and in a preliminary study, we found that intestinal inflammation to the rats increased exit of ILC3 into mesenteric lymphatic vessels. We established observation system of the migration of ILC from intestinal mucosa to mesenteric lymph nodes by using rats with mesenteric lymphadenectomy (MLNx). We aimed to examine how intake of IN affects migration of ILC.

Method: Wistar male rats (5 weeks) received MLNx. Around 5 weeks after MLNx, thoracic duct was cannulated for collecting lymphatic fluid. IN was fed via tube inserted into duodenum from the next day. Lymphatic fluid was collected before and after infusing IN and analyzed by flow cytometry to investigate the ILC composition.

Result: After administering IN, percentage of ILC among all lymphocytes did not change, but proportion of ILC3 among total ILC was significantly increased (15.0% to 41.1%). Immature ILC was decreased from 37.8% to 19.0% by IN, suggesting that immature ILC differentiated to mature ILC, especially to ILC3.

Conclusion: Recently, accumulating evidence has indicated AhR activation is necessary for secretion of IL-22 by ILC3s. This result indicates that IN might induce differentiation of ILC3 and contribute to enhance intestinal immunity.

Y-03 | NLRP3 Inflammasome-derived interleukin-1β attenuates stress-induced gastric injury via the COX-2/PGE_2 axis

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Backgrounds and Aims: The inflammasomes promote pro-caspase-1 cleavage, leading to pro-interleukin (IL)-1β maturation. We investigated the role of IL-1β and NLRP3 inflammasome in stress-induced gastric injury by water-immersion restraint stress (WIRS).

Methods: After fasting for 24 h, male wild-type, caspase-1-knockout (KO), NLPR3 KO and Toll-like receptor 4 (TLR4) KO mice were placed in a restraint cage and immersed in the water bath. Mucosal lesions were subjected to measurements of ulcer size and assay of mRNA levels by real-time RT-PCR. Protein levels of mature IL-1β and cleaved caspase-1 in stomach were measured by Western blotting. Prostaglandin E_2 (PGE_2) production by gastric tissue was assessed by enzyme immunoassay.

Results: WIRS induced gastric injury with an increase in proinflammatory cytokine expression. Induction of gastric injury was also associated with increases in COX-2 expression and PGE_2 synthesis and promotion of NLRP3 inflammasome activation and IL-1β maturation. Exogenous IL-1β attenuated the injury and positively regulated COX-2 expression, whereas the anti-IL-1β antibody exerted the opposite effects. NLRP3-/- and caspase-1-/- mice exhibited severe injury and no increase in IL-1β maturation or COX-2 expression, and this aggravation of the injury was abolished by exogenous IL-1β supplementation. Administration of PGE_2 also rescued the phenotype of the two knockout mice and prevented overexpression of inflammatory cytokines after WIRS challenge. Toll-like receptor 4 (TLR4)-/- mice were hyporesponsive to WIRS in terms of mature IL-1β production.

Conclusion: These results suggest that NLRP3 inflammasome-derived IL-1β plays a protective role in stress-induced gastric injury via activation of the COX-2/PGE_2 axis and that TLR4 signaling may be involved in NLRP3 inflammasome activation.

All experimental procedures were approved by the Animal Care Committee of Osaka City University Graduate School of Medicine (Approval number 18009).

Y-04 | NLRP3 inflammasome-mediated caspase-1 activation prevents gastric mucosal healing

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Background and Aim: Inflammasome is new concept for explaining inflammation system and its activation permits processing of pro-caspase-1 into cleaved caspase-1, which promotes the processing of pro-IL-1β into mature-IL-1β. The aim of this study is to evaluate whether NLRP3 inflammasome is involved in gastric ulcer healing.

Methods: Male wild-type, caspase-1-knockout (KO) mice were used. Gastric ulcers were induced by serosal application of acetic acid. Mice were sacrificed on day 4, 6 or 9 of ulcer induction, and ulcerous tissues were subjected to measurements of ulcer size, immunohistochemical examination, and assay of mRNA levels by real-time RT-PCR. Moreover, the mice were intraperitoneally administered caspase-1 inhibitor (Ac-YVAD-CHO), NLRP3 inflammasome inhibitor (Isoliquiritigenin) or vehicle beginning at 4 days after ulcer induction. All experimental procedures were approved by the Animal Care Committee of Osaka City University Graduate School of Medicine.

Results: Ulcer size peaked on day 4 and the healing phase of the experimental gastric ulcer started on day 4 after ulcer induction. The expression of TNFα), IL-1β and NLR family pyrin domain-containing 3 (NLRP3) mRNA also reached a maximum on day 4 and decreased over time thereafter. Immunohistochemically, NLRP3 and caspase-1 proteins were detected mainly in inflammatory cells such as macrophages in ulcerous areas and in some epithelial cells. Genetic deficiency of caspase-1 markedly enhanced ulcer healing with TNF-α and IL-1β mRNA reduction by 56.5% and 66.0%, respectively. Administration of Ac-YVAD-CHO at a dose of both 0.1 and 1 mg/kg significantly enhanced ulcer healing with reduction of TNF-α (53.1% and 68.0%, respectively) and IL-1β (52.2% and 76.6%, respectively) mRNA expression. Administration of NLRP3 inflammasome inhibitor (Isoliquiritigenin) also enhanced ulcer healing.

Conclusion: These results suggest that NLRP3 inflammasome-mediated caspase-1 activation prevents gastric mucosal healing and inhibiting caspase-1 activity might be a novel strategy to enhance mucosal healing.