endothelial cell fibers would be a fundamental tissue unit for constructing three-dimensional (3D) macroscopic tissues with vascular network and for analyzing cellular behaviors (ex. vasculogenesis or angiogenesis) under intercellular/chemical/mechanical/fluidic interactions.

1PT226 分子ロボットによる人工バンスペルミア

Artificial Panspermia by Molecular Robots

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Molecular robotics is an emerging research field aiming at development of a systematic methodology to design complex molecular machines, which have a potential to overcome difficult problems that natural living system cannot solve. Recently, owing to the rapid progress in DNA nanotechnology, many types of molecular devices have been proposed. Those devices, usually made of several artificially synthesized DNA, have shapes and functions designed by humans. However, these devices work only in test tubes. Extending their operation to the wider conditions, such as in vivo, in low temperature and in high/low pH, is one of the important research targets of molecular robotics. An example of such extreme environment is the space, where not only the zero pressure but intensive radiations surround the molecules. Here, we think artificial panspermia* is a good target for molecular robotics, and focus on environmental resistance of molecular devices against cosmic radiation and freeze-dry treatment. As a test piece, we have designed a DNA nanostructure and evaluated its functionality (in this case, the shape) after re-hydration. Atomic force microscopy observation showed that the structure well maintains the designed shape throughout the treatment. *"Panspermia" is a hypothesis about the origin of life, assuming interstellar travel of living organisms.

1PT227 細胞膜上の分子ロボットの作成に向けて

Prototype molecular robots working on cellular membrane

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Recent progress of micro-nano technology enables us to design "molecular robots" [1,2,3]. Molecular robot is a system of molecular devices in which DNA nanostructures, DNA devices and modified proteins are combined as a coordinated system. They are in the scale of up to several tens nanometer, and huge number of the same robot can be produced in a test tube by self-assembly. We are investigating interaction between such molecular robots and living cells. In the operations of the molecular robots, the following features are important: 1) target cell specificity and 2) launching/landing back to/from the target. Here, we present a design of prototype robot working on cellular membrane. The robot has hydrophobic "legs" that holds onto the membrane. Because of diffusion of lipid molecules in the membrane, the robot is also expected to show lateral diffusive behavior. To verify this, single molecular tracking experiment with fluorescent-tagged robot has been conducted. The data obtained by total internal reflection fluorescence (TIRF) microscopy clearly showed lateral diffusion of the robot in several seconds. In the future applications, these robots floating on the membrane will protect cells from virus infection, or will be used to control the specific receptors on the cell.

[1] G. M. Church et al. *Science* 2012, 335, 831-834 [2] W. Winfree et al. *PNAS* 2012, vol.109, no.17, 6405-6410 [3] H. Sugiyama et al. *JACS* 2010, 132, 1592-1597

1PT228 分子ロボティクス：リモコン分子クラウラー・プロトタイプの構築

Molecular robotics: Construction of a remote control molecule crawler prototype

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Molecular robotics is a new research field to develop an artificial molecular system. The major methodology, self-organization of biomacromolecules, is quite suitable for biological applications. Thus, designing smart drug delivery system (DDS) is strongly expected in the future. Here we aim to create a "remote-controllable" molecular system. We constructed the prototype with water-in-oil droplet (10 to 50 micrometer in diameter), incorporated magnetic nano particles. The magneto-droplets showed three-dimensional motion induced

by external magnetic field. It was predicted that the accumulation pattern of the magnetic particles is strongly affected by the whole droplet movement. The droplet can easily transfer the structure into giant liposome by passing through the lipidic oil/water surface. Thus, we are now designing a "movable" molecular system on using this chassis. The result will be reported.

1PT229 バクテリア駆動マイクロギアの構築とトルク計測

Building and torque measurement of bacteria driven micro gear

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Bio-hybrid system has been attracting attention in recent years. The biological macromolecule and living organism are worked as very small and highly effective actuator and these bio-actuators are expected as next generation motivity. Especially, several bio motors were reported. However, the research field of bio-actuator is in advance, many unsolved problems exist. For example, biological reaction is not well known, evaluation of bio actuator is not enough, therefore developing control method for such kind of reaction is important. In this study, to realize the new bio motor, which can be applied to micro-nano systems power source (built-in mechanical factor), we used *Vibrio alginolyticus*, some kind of marine bacteria, specific motile mode "Surface swarming" as actuator. At first, we tried to drive micro beads on surface swarming of *V. alginolyticus*. Furthermore, we succeeded in controlling movement of surface swarming by ratchet shape micro channel and then driving the micro gear in the closed micro channel. As a next step, to transmit power of rotational movement, we fabricated the open micro channel and micro object (floating pillar, micro gear etc.) and succeeded in driving the object in the open micro channel. In addition, we revealed that rotational power of floating pillar by deformation of nano probe. Finally, we assembled the micro gear with shaft like crank. Therefore, it became possible transmitting the power from rotational movement to outside. Thus, we achieved to construct base of bio-motor.

1PT230 Evaluation of microtubule deformation by live cell imaging and image analysis

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Deformation of cytoskeleton is thought to be a converter of mechanical stimulus into chemical activity in living cells. Although recent reports showed that actin fiber, one of cytoskeleton, acts as a converter, contribution of other cytoskeletons is still unclear. As a first step to elucidate this question, we began to evaluate deformation of other cytoskeletons.

We evaluated microtubule deformation by live observation of GFP-tagged tubulin expressing cells and image analysis. We observed microtubules dynamically bent in contrast to straight actin fiber. Therefore, longitudinal deformation of microtubule is difficult to evaluate by using actin fiber deformation analysis. To overcome this problem, we developed image analysis software aimed to extract fluorescent intensity profile along curved microtubule and identify the same points on the fiber from a set of time course images. Microtubule deformation was evaluated by time change of distance between the identical points. We successfully evaluated deformation of curving microtubule. The next step of our study is to evaluate validity of our analysis. In this annual meeting, we will also report microtubule deformation under cell deformation. Besides, we will discuss role of microtubule deformation as a cellular sensor.

1PT231 マイクロファイバークラウドマトリックスの弾性パターンニングによる三次元細胞運動のメカニカル制御

Mechanical control of 3-D cell movement in elastically-micropatterned matrix of micro-fiber gels

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The cell movements are critically affected by the stiffness of extracellular surroundings of 2-D substrate surfaces or 3-D matrices. To scrutinize the mechanobiology of the cell motility, systematic design of the extracellular mechanical surroundings is quite essential. In this study, we have developed the novel methodology of microelasticity-design of 3-D cell culture matrix which consists of nonwoven mesh of swollen micro-fibrous gels with well-tuned different elasticity, in order to clarify the effect of matrix stiffness on the 3-D cell movement. Nanofiber mesh of photocurable styrenated gelatin (StG)

including radical generator camphoquinone was prepared by electrospinning, cross-linked in ethanol media, and swollen in aqueous solution, which successfully produced swollen micro-fiber gel matrix. By regulating the photocuring conditions, elastic modulus of fiber gel sheet was well-tuned. Assessment of movement of 3T3 fibroblasts on the prepared gel sheets with different stiffness showed that cells extended well on the surface of the sheet and did not enter the inside of soft matrix (1.4 kPa), whereas cells got into the hard sheet (15.8 kPa) and finally reached to the bottom of the sheet 6 hours after seeding. This result suggested that 3-D cell mechanotaxis can be driven by matrix-stiffness dependent manner as well as the 2-D mechanotaxis. Mechanism of such 3-D mechanotaxis and possible manipulation of 3-D cell movement by the design of stiffness of gel sheet are discussed.

1PT232 微視的弾性境界の曲率設計による細胞メカノタクシスの高効率誘導
Efficient manipulation of cell mechanotaxis: effect of the curvature of micro-elasticity boundary

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The directional cell movements induced by a mechanical gradient on a substrate surface, so-called mechanotaxis, are useful model phenomenon to investigate the mechanobiology of cell motility. So far, we have established the elasticity boundary conditions required to induce mechanotaxis of fibroblasts with certain reproducibility; sharp linear boundary with enough narrow width and enough high elasticity jump in the single-cell adhered interface on cell culture gels. However, the induction efficiency of mechanotaxis was significantly affected by the shape of moving cells, suggesting the importance of a shape interaction between cells and elasticity boundary. In this study, to assess the effect of the shape interactions on the induction efficiency of mechanotaxis, a curvature of the elasticity boundary was systematically designed with a custom-built mask-free photolithography using elasticity-tunable photocurable styrenated gelatin. Fixing the elastic gradient strength as 30-40 kPa/ 50 μ m, the radius of curvature of boundary was designed as 50, 100, 250, 500, 750, and 1000 μ m in both convex and concave shapes. Migration of 3T3 fibroblasts was observed on these boundaries and efficiency of induction of mechanotaxis was analyzed. As the result, highly efficient mechanotaxis was induced on the convex boundary with 100 μ m in radius and on the concave boundary with 750 μ m in radius. Interestingly, directional cell migration toward a softer region emerged on the concave boundary with 50 μ m in radius for the first time.

1PT233 線虫内部への蛍光ナノビーズの選択的導入
Selective Injection of Fluorescent Nanobeads into *Caenorhabditis elegans*

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We present a novel selective injection of fluorescent nanobeads into *Caenorhabditis elegans* (*C. elegans*) based on bio-nanomanipulation technique for in-vivo single cell analysis.

The nanomanipulation system was constructed under hybrid microscope. The hybrid microscope consists of the optical microscope (OM) and environmental-scanning electron microscope (E-SEM). It realizes detail analyses of biological specimens by fluorescent imaging by the OM, and nano-scale manipulation by the imaging of the E-SEM.

Based on the bio-nano manipulation system, we propose a nanoinjection method by the nanoprobe insertion for embeddedness of nanobeads. The nanoprobe was designed to have the four tips to fix single nanobead. It was fabricated by focused ion beam (FIB) process. The nano-scale probe size is considered to be important for minimal damage to the biological specimens.

In this work, as a biological specimen, the *C. elegans* was used, because it is one of the important model organisms for various biological analysis. The nanoinjection technique was applied to embed a fluorescent nanobead into a specific cell inside *C. elegans* for in-vivo analysis. We inserted the nanoprobe into the body of *C. elegans* at a depth in approximately 6 μ m. The size of fluorescent nanobeads was 500 nm in diameter. The proposed system is considered to be important as future nano-surgery system for life innovation using model organism.

1PT234 W/O ドロップレット内での酵母を用いた G タンパク質共役受容体のリガンドアッセイシステム

Yeast-based ligand assay system for detecting G protein-coupled receptor activation in water-in-oil droplets

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G protein-coupled receptors (GPCRs) are involved in many different cellular processes, and are targets of many therapeutic drugs. We developed a yeast-based ligand assay system for detecting GPCR activation in water-in-oil (W/O) droplets. Our system is based on linking between the genotype and the phenotype using W/O droplets. DNA library encoding peptides, in vitro transcription-translation system, and budding yeast cells expressing target GPCR are co-encapsulated into droplets using a microchannel. Yeast cells are genetically engineered to express GFP in response to ligand stimulation. The generated droplets were collected into a microcentrifuge tube and incubated to allow the gene translation and ligand stimulation. The droplets would be fluorescent when synthesized peptides stimulate GPCRs, enabling easy identification of ligand candidates. Using this system, we firstly demonstrated cell-free GFP synthesis in the presence of yeast cells in W/O droplets, indicating the presence of yeast cells did not inhibit protein expression. Next, we encapsulated yeast cells expressing Ste2p (an endogenous GPCR) and the gene of α -factor (ligand of Ste2p) in the droplets. After incubation, GFP-expressing cells were clearly observed in response to α -factor stimulation. The α -factor synthesized from the five-molecule DNA template in the droplets successfully induced GFP expression. Our system will provide an effective approach for the high-throughput screening of peptide ligands targeted at mammalian GPCRs by evolutionary molecular engineering.

1PT235 フロー型乳酸バイオセンサの開発とその脳内乳酸測定への応用
Development of a flow-type lactate biosensor and its application to the measurement of lactate in the mouse brain

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Lactate biosensors which enable us to measure lactate concentration in blood and other body fluids are applicable to some medical fields such as sports medicine. We fabricated a lactate biosensor by a flow-type sensing system, in which the injected sample flows into an immobilized enzyme (lactate oxidase; LOD) column. The column was fabricated by packing an immobilized enzyme support into a 30 mm long column. The support was obtained by immobilizing LOD on silica particles (particle size: 30-60 mesh). The immobilization was performed by loading LOD onto the aminoalkyl-bonded particles with glutaraldehyde. The column was kept at a constant temperature (37°C). The generated H₂O₂ or consumed O₂ in the column was detected by the H₂O₂ electrode or O₂ electrode. The fabricated biosensor could measure lactate concentration up to 10 mM. By using it, we next measured lactate levels of the mouse brain. The samples were prepared by homogenizing the brain slices in ACSF. The results showed the differences of lactate levels among each part of the brain and among each age of the mouse.

1PT236 マイクロチャンバー内での β -グルコシダーゼ 1 分子の酵素活性のモニタリング

Monitoring the single-molecule enzymatic activity of β -glucosidase in a microchamber array chip

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β -glucosidase (BGL), which catalyzes the hydrolysis of cellobiose to glucose, is one of the important enzymes in bioethanol production. We aim to obtain BGL mutants with improved hydrolytic activity by directed evolution. To screen many potential mutants easily and precisely, we are trying to develop the single-