PLS regression. Finally, we obtained four principal component dimensions characterizing characterize some phenotypes. This data driven approach is effective to identify undefined aspects of complex temporal signal transduction networks.

1PT215 幹細胞様のふるまいを示す人工遺伝子ネットワークの構築

Synthetic biology of stem cells: creating artificial gene network of "differentiation"

Sumire Ono¹, Reiko Okura¹, Yuichi Wakamoto^{2,3} (¹Grad. Sch. Arts and Sci., Univ. Tokyo, ²Research Center for Complex Systems Biology, Univ of Tokyo, ³JST PRESTO)

Stem cells can differentiate spontaneously and irreversibly, producing a variety of differentiated cells in context-dependent manners. It remains to be resolved what kind of fundamental characteristics of intracellular network enable such remarkable attributes of stem cells. Recently, Goto and Kaneko have proposed a dynamical systems model of stem cells that can explain spontaneous bifurcation of cellular states using two-gene regulatory network. In this model, a "diffusible inducer" causes cellular interaction, leading to coexistence of two cellular states. The simplicity of the regulation proposed in the model should allow us to synthesize such regulatory network artificially and thereby test the hypothesis regarding the stem cell gene networks.

Inspired by this theoretical study, we aim to construct an *Escherichia coli* strain that possesses an artificial gene regulatory network equivalent to the scheme presented in the model. To realize cellular interaction through diffusible inducer, we utilized the lux operon of *Vibrio fischeri*. However, the original lux operon has positive regulation whereas the inducer acts negatively in the proposed model. For this reason, we designed the actual network with three genes instead of two in a manner that tet operon mediates the lux operon's function. T7 RNA Polymerase system and the lac operon were also used for regulation. RFP and YFP were also introduced as reporters for the activities of the sub-networks. The results and the perspective will be discussed in this meeting.

1PT216 カタウレイボヤ初期胚におけるラマン顕微鏡による細胞内分子の検出

Detection of bio-molecules localized in specific cells of *Ciona intestinalis* embryo by Raman spectroscopy

Mitsuru Nakamura, Kohji Hotta, Kotaro Oka (Graduate School of Science and Technology, Keio University)

Localization of bio-molecules plays central roles in the specification of cell fates and morphogenesis in the embryogenesis. For this reason, to trace the distribution of bio-molecules, especially, mRNAs and proteins, is one of the major issues in developmental biology. In current study, genetic manipulation can be used to observe molecular distribution through the use of fluorescein-labeling molecules. This technique, however, can result in phenotypic changes, need prior identification of target molecules, and the number of labels applicable is limited. Raman spectroscopy, in contrast, is a low-invasive and "label-free" technique, allowing imaging of the distribution of the number of bio-molecules, including molecules without prior identification. In this study on the *Ciona intestinalis* embryogenesis, we explore the application of Raman spectroscopy within the developmental process to investigate the distribution of bio-molecules without labeling. We applied Raman spectroscopy to collect the spectra scattered from *Ciona* embryo at single-cell resolution for early developmental stages. After the spectral analysis, we succeeded to detect vegetal hemisphere-specific localization of some bio-molecules and its localization is similar to maternal factors. This result suggests that Raman spectroscopic imaging in low-invasive and label-free manner on the spatial distribution of bio-molecules is feasible to investigate for developmental studies in *Ciona intestinalis*.

1PT217 細胞分化のエピジェネティック動力学の平均場理論

Mean field theory of epigenetic dynamics of cell differentiation

Takaya Koshii¹, Tomoki P. Terada², Masaki Sasaki² (¹Department of Applied Physics, Nagoya University, ²Department of Computational Science and Engineering, Nagoya University)

Expression of the marker genes of cell differentiation is regulated by the epigenetic modifications of histones, and such epigenetic modifications are regulated by the expression of genes which encode transcription factors (TFs). We analyzed this entangled dynamics of gene expression and epigenetic modification by developing a mean field model of the simplified gene network. In

this model the network of three genes is assumed; the network is composed of gene A which maintains the pluripotent state, gene B which induces the differentiation to one lineage, and gene C which induces the differentiation to the other lineage. Each TF synthesized from each of gene A, B, and C is assumed to mutually represses the expression of other genes. We analyze how the difference between the rate of the TF binding/unbinding and the rate of the histone methylation/demethylation affects the process of transitions among the cell states.

1PT218 細胞分化の力学系モデル：安定な発生過程と細胞リプログラミングの理解へ向けて

A dynamical system modeling of cell differentiation for understanding of robust development and cell reprogramming

Chikara Furusawa¹, Kunihiro Kaneko² (¹QBiC, RIKEN, ²Dept. Basic Sci., Univ. Tokyo)

Two important problems in stem cell biology are how to characterize the difference in cellular states between undifferentiated stem cells and terminally differentiated cells, and how to understand the dynamics of cell reprogramming from terminally differentiated cells to undifferentiated cells. In this study, to answer these questions, we have carried out a dynamical systems modeling of interacting cells with a gene regulatory network to reveal stem cell differentiation triggered by the cell-cell interactions. We have simulated cellular dynamics using gene regulatory networks with a small number of genes (e.g., less than 10), and screened regulatory networks that can generate cell-type heterogeneity through cell-cell interaction by using genetic algorithm simulations. The results revealed that expression dynamics of cells that have potential to differentiate (i.e. stem cells) generally show irregular (chaotic) oscillation, whereas such complex oscillation is lost for terminally differentiated cell types. Furthermore, we found that, to reprogram terminally differentiated cells to undifferentiated cells, a perturbation to genes which are positively auto-regulated by its products is important. The emergence of stable cellular states that are not chosen by normal development and its relevance to cancer cells will also be discussed.

1PT219 リバーシブル・ネットワーク・リコネクションモデルによる形態形成過程の組織大変形シミュレーション

Simulations of large deformation during tissue morphogenesis using reversible network reconnection model

Satoru Okuda¹, Yasuhiro Inoue¹, Mototsugu Eiraku², Yoshiki Sasaki², Taiji Adachi¹ (¹Inst. Frontier Med. Sci., Kyoto Univ., ²Riken CDB)

Tissue morphogenesis is accompanied by large three-dimensional (3D) deformations, in which mechanical interactions among multiple cells are spatiotemporally regulated. To reveal the deformation mechanisms, in this study, we newly propose the reversible network reconnection (RNR) model. The RNR model is developed on the basis of 3D vertex models, which express a multicellular aggregate as a network composed of vertexes. 3D vertex models have successfully simulated morphogenetic dynamics by expressing cellular rearrangements as network reconnections. However, in cases simulating large deformations during dynamic tissue morphogenesis, a 3D vertex model can possibly induce unphysical results and failures. We found this is because of geometrical irreversibility, energetic inconsistency, and topological irreversibility in network reconnections. Mathematical and numerical analyses suggested that these problems can be resolved in the RNR model. In addition, to demonstrate the applicability of the RNR model, we simulated tissue deformation of growing cell sheets and showed that the proposed model can simulate large tissue deformations, in which large changes occur in the local curvatures and layer formations of tissues. Thus, the proposed RNR model enables *in silico* recapitulation of complex tissue morphogenesis.

1PT220 細胞体位置と神経突起の伸長方向を制御した3次元脳回路再構成技術の開発

Development of Three-dimensional reconstruction brain circuit controlling the direction of neurite elongation and cell body position

Aoi Odawara, Ikuro Suzuki, Masao Gotoh (Tokyo University of Technology)

3D reconstruction techniques in biological tissues have been expected to be beneficial in validation of drug candidates and an effective transplant technology in regeneration medicine. Recently, 3D reconstruction techniques using cells combined with biocompatible materials have been developed. In

particular, cell sheet transplantation techniques applied to heart and retina. However, the 3D reconstruction of biological tissues that has an orientation as the brain nerve tissues is still not developed. The aim of this study is to develop a 3D reconstruction technique of brain circuit, controlling the spatial arrangement of neuronal cells. We have developed a 3D neuronal circuit culturing technique by controlling the position of nerve cell body and the direction of neurite elongation using collagen fiber orientation techniques and poly dimethylsiloxane (PDMS) micro-processing. As a result, we observed that the neurite grow in one direction along the collagen fiber with cell body position fixed. In addition, by observing neurite configuration in Z axis using confocal microscope, we confirmed that neurite in 3D gel matrix are elongated in one way. Furthermore, a multilayer structure that mimics the six-layer structure of the cerebral cortex was successfully created. We were able to confirm that neurite are elongated in one way among three layers. Finally, to confirm the function of the 3D reconstructed neuronal circuit, we were able to obtain the propagation of action potential between two cell body layers using multielectrode arrays (MEAs).

1PT221 3次元ネットワーク再構成のための神経組織の脱細胞化条件
Decellularized conditions of neuronal tissue for the reconstruction of 3D neuronal network

Shota Amano, Ikuro Suzuki, Masao Gotoh (Tokyo University of Technology)

Artificial heart and joints has been translated into the medical field. In recent years, transplantation techniques used by these artificial tissues have been expected. Cell transplantation using cell sheet such as in epidermal tissue, corneal epithelium, and the heart, has been successful. The discovery of iPS cell made it an important tool in creating a variety of tissues, but in cell culture, the use of decellularized cellular components such as scaffold has been of interest because of its ability to keep the three-dimensional tissue structure and extracellular matrix (ECM) stable. Until now, changes in decellularization conditions and ECM evaluation in liver, lung, heart tissues, and muscles has been investigated, but not in nerve tissue. Therefore, the aim of this study is to investigate the changes in decellularization conditions and ECM evaluation of nerve tissue. Using rat brain, nerve tissue decellularization was performed using de-sodium dodecyl sulfate (SDS), Trypsin/EDTA, and TritonX-100, decellularized tissues were stained using hematoxylin and eosin (H&E). In addition, the processing of each decellularized nerve tissue's ECM was evaluated. Together all the results of nerve tissue decellularization, will be reported at the conference.

1PT222 一分子蛍光ソーティングを目指したマイクロ流体システムの構築
Construction of a microfluidic system for fluorescence-based single-molecule sorting

Sho Saitoh¹, Hiroyuki Oikawa², Kiyoto Kamagata², Satoshi Takahashi²
 (¹Grad. Sch. Life Sci., Tohoku Univ., ²IMRAM, Tohoku Univ.)

Techniques of sorting micro-particles such as cells have found wide applications in biological sciences; however, sorting of biological molecules with relatively small molecular weights including proteins and nucleic acids is still difficult. This is mainly because of the lack of the method to discriminate molecules sensitively at the single molecule level. We are currently constructing a fluorescence-based single-molecule sorting device, which is composed of an optical system for the detection of fluorescence from fluorophores labeled to sample molecules and the microfluidic system for the sorting of molecules after the fluorescence detection. As the optical system, we utilized a light correction system based on a spherical mirror, which achieves a large detection area and high numerical aperture at the same time. As the microfluidic system, we constructed a silica-based chip, in which an inlet channel and two outlet channels for waste and collection were carved. The sample was introduced to the inlet channel, and its fluorescence intensity was detected at the observation point. Based on the detected fluorescence intensity, the flow was controlled to direct sample either to the waste channel or the collection channel. We utilized miniature solenoid valves to switch the sample flow. We confirmed that the system can be used to sort a mixture of two kinds of microbeads with different colors. To further examine the performance of the developed system, we are currently conducting sorting experiments of DNAs with different lengths.

1PT223 電子顕微鏡画像分類の利便化のためのツール開発
Development of a convenient tool for making processes of particle clustering and averaging in 3D reconstruction from electron micrographs

Tatsuya Kihara, Takuo Yasunaga (Dept. of Biosc. and Bioinfo., Grad. of Comp. Sc. and System Eng., Kyushu Inst. of Tech.)

Eos (Extensible object-oriented system for macromolecular imaging), we have been developing, has many tools to process electron micrographs and environment for software development. Some tools provide GUI (Graphical User Interface)-based one in Eos but others not. A group of program for classification and averaging has been also implemented as only CUI (Character User Interface)-based ones. Therefore, we tried to develop a convenient tool with GUI. This was implemented by an integration of different programs under Eos and so only a few simple steps using mouse were necessary for processing to be executed. I succeeded in developing the tool with GUI and it was evaluated in actual analyses by some researchers. At the last meeting, we reported the evaluations of the tool using model data sets, phantoms. We here report evaluations for actual electron micrographs in these analyses. Actually, the tests showed some problems from the researchers such as limits of image number, no function for resetting zooming size and so on. At present we solved some of the problems, added to new functions and modified the tool to be more convenient. However some still remain. Furthermore, we try to test the tools iteratively and add functions and parameters for more precise image analysis and refinement.

1PT224 高重力を利用した非球形構造を持つマイクロハイドロゲル粒子の作製法

High-gravity-based synthesizing method for hydrogel microparticles with non-spherical structures

Masayuki Hayakawa¹, Hiroaki Onoe³, Ken H. Nagai⁴, Masahiro Takinoue^{1,2}
 (¹Interdisciplinary Grad. Sch. Sci. and Eng., Tokyo Tech., ²PRESTO, JST, ³IIS, Univ. of Tokyo, ⁴Dept. Phys., Univ. of Tokyo)

In recent years, hydrogel-based anisotropic microparticles have attracted much attention due to their various applications including micrometer-scaled self-assembly, tissue engineering, drug delivery, and artificial cell models. By three-dimensionally organizing such anisotropic microparticles encapsulating some functional molecules, it will become possible to construct self-assembled systems with higher-order functions as living cells and living tissues. However, constructing such three-dimensional structures from anisotropic microparticles is now difficult because it is unable to synthesize three-dimensionally complex anisotropic particles such as colorful beach balls, gears, etc. due to the use of two-dimensional microfluidic channels in the synthesis of microparticles. Thus, it is desired to develop a method to generate three-dimensionally anisotropic microparticles.

In this presentation, we report a method for generating three-dimensionally anisotropic microparticles such as hemispherical microparticles. This method was based on Centrifuge-based Droplet Shooting Device (CDSD) [1]; it is a device to synthesize monodisperse microparticles with multiple compartments. Here, we constructed three-dimensionally anisotropic microparticles by removing a part of the multiple compartments of the microparticles. We believe that our method will contribute to the study of more complex self-assembled systems of microparticles.

[1] Maeda et al., Adv. Mater. (2012)

1PT225 ハイドロゲルマイクロファイバー内での血管内皮細胞の管構造形成
Tube Formation of Endothelial Cells in the Hydrogel Microfiber

Hiroaki Onoe^{1,2}, Shoji Takeuchi^{1,2} (¹IIS, The University of Tokyo, ²ERATO Takeuchi Biohybrid Innovation Project, JST)

Creating vascular network in a macroscopic artificial tissue has been stated as an essential element for constructing long-life artificial tissues having nutrient/metabolite circulation. Various attempts have been made to create blood vessels in an artificial tissue though, the arrangement of vascular network in a designed 3D configuration have still been a challenging issue.

Recently, we have proposed a cell-encapsulating core-shell hydrogel fiber, named "cell fiber". One important feature of our "cell fiber" is that we can freely manipulate the fibers with keeping cellular activities and assemble them into designed 3D configurations. Here we present the tube formation of endothelial cells in the collagen/alginate core-shell hydrogel microfiber. We found that encapsulated MS1 (mouse pancreatic endothelial cell line) and HUVEC (human umbilical vein endothelial cell) spontaneously create tube-like monolayer cell structures along the axial direction of the fiber at meter-length scale. Because the produced endothelial tubes are free-standing and can be manipulated in a culture medium with the support of the alginate shell, our