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

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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

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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

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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

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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

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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