- 1P306 微細加工基板上をミオシンによって駆動されるアクチン運動のシミュレーション
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Computer Simulation of Actin Filament Movements Driven by Myosins in Microfabricated Patterns
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There have been attempts to utilize myosin/actin and kinesin/microtubule as molecular shuttles into Lab-on-a-chip devices. Molecular shuttles are cytoskeletons loaded with cargo and propelled by associated motor proteins along predetermined tracks to deliver the cargo to specific destinations. Both molecular shuttles have their own unique advantages and disadvantages due to the intrinsic properties of the motor proteins and the cytoskeletal filaments. For example, a myosin/actin-based molecular shuttle can glide faster but needs narrower guiding tracks than a kinesin/microtubule-based one. Hence, depending on specific tasks of devices, appropriate molecular shuttles should be selected and integrated with appropriate guiding module designs. In search for appropriate module designs for kinesin/microtubule-based molecular shuttles, a computer simulation was shown to be useful. However, there was no computer simulation for myosin/actin-based molecular shuttles. To enable such a simulation, we measured in vitro motility assays the required parameters, which were time-averaged velocity, motional diffusion coefficient and persistence length of actin/myosin-based molecular shuttles. Our computer simulation implemented with the parameter values reasonably reproduced experimental results reported by other groups. Use of our computer simulations for both myosin/actin- and kinesin/microtubule-based molecular shuttles enables one to select appropriate molecular shuttles and module designs in silico.

- 1P308 高サンプル流速下における画像認識型セルソーターの性能改善
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Improvement of microscopic-image recognition of on-chip cell-sorting system under high flow rates of sample particles
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For phenotyping and studying the functions of cells, we need to purify target cells from a crude mixture of various kinds of different cells. We have developed a series of on-chip cell-sorting system that based on the real time recognition of microscopic images of cells. A Y-shaped microfluidic pathway on a plastic chip was equipped with a pair of gel electrodes at its branch point. Sample cells flowing through the pathway run into one of the downstream branches without any voltage between the electrodes. When a target cell was recognized by an ultra-high-speed camera-based image-recognition unit, the cell was sorted into the other branch through the application of a direct-current voltage between the electrodes. In this meeting, we tried to improve the performance of the cell sorting system at high flow rates of a sample flux. The flow rate was controlled accurately with a wide dynamic range from 1 to 1600 mm/sec using pressure regulators instead of syringe pumps. At high flow rates, the images of a fast moving subject become blurred by afterimage. To acquire crisp images, we used a short-pulsed light illumination (~1 μsec) of LED and a high-speed (1000 frame/sec) CCD camera. The optimum voltages between gel electrodes were examined at various velocities of the sample flux. For example, latex beads (2 μm in diameter) were sorted into the target branch using electric pulses of 45V at a high-flow rate (200 mm/sec). We will discuss the further detailed results and discussion in the meeting.

- 1P307 微細加工技術を用いたオンチップ多種細胞同時精製システムの開発
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Development of on-chip on-time multi cell sorting system exploiting microfabrication technology
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Cells are thought to be a minimum unit to keep their epigenetic recording and functions and thus, are thought to have huge variety of phenotypes even within a single genotype cell sources. Thus the technology to purify cells without damaging them is thought to be essential for cell-based research and screening. In the last meeting, we reported a on-chip cell sorting system based on the recognition of microscopic image of cells. However, this sorting system could separate only two types of cells, because it had Y-shaped two pathways in the separation area. In this report, to sort various types of cells from crude culture, we devised a new sorting chip which could separate several types of cells individually on the same chip. We designed and fabricated PDMS sorting chip applying microfabrication technology as follows: A linear flow channel (50 μm in width, 25 μm in depth) had several pockets as cell storages; each pocket was connected to a agarose-gel electrode for capturing different types of cells individually by electrostatic force. We confirmed that polystyrene beads (diameter: 2 μm) flowing through the channel got caught in a pocket when DC voltage was applied between the gel electrodes beside the pocket. In addition, the caught beads in the pocket were released into the flow channel by reversing the electric field. The detailed results and discussion will be presented in the meeting especially about the purification technology in which particles are sorted into separate pockets by controlling the voltage impressing.

- 1P309 3次元マイクロ流路を用いた微生物の均一化マイクロゲルカプセル化
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Formation of Monodisperse Alginate Hydrogel Capsules with Microbes using Three-Dimensional Microfluidic Axisymmetric Flow-Focusing Device
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We present a simple method to form monodisperse alginate hydrogel capsules with microbes using a monolithic three-dimensional microfluidic axisymmetric flow-focusing device (3D AFFD). The 3D AFFD is fabricated with stereolithography and we can form axisymmetric orifices of the microsize. In the 3D AFFD, the fluid is focused and broken into droplets at the narrow orifice. By confining droplets to the central axis of the microchannel, we avoid the problem of wetting even when droplets with microbes are produced. To gelate these microbe-encapsulating droplets, we employed the internal gelation method. This method involves the formation of Na-alginate droplets containing CaCO₃ nanoparticles in corn oil. Upon pH reduction when acetic acid is introduced, Ca²⁺ ions are released from the CaCO₃ nano-particles, crosslinking the alginate to form a hydrogel. This method allows the formation of well-defined and homogenous alginate hydrogel beads with ease. By combining the 3D AFFD with internal gelation method, microbes are successfully encapsulated in monodisperse alginate hydrogel beads. We believe these microbe-encapsulating capsules can be used as bioreactors and will be useful for applications ranging diverse fields such as in the pharmaceutical, food and biotechnology industries.