Kinesin-based transportation of oil droplets along microtubules for applications in nanotechnology

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Host Professor: H. FUJITA

Keywords: biomolecular motors, kinesin, microtubule, cargo, transportation

Context

Scaling laws governing fluid flow and diffusion-based transport indicate that biomolecular motors are an effective option for actively transporting molecules/particles in nanodevices. Kinesin proteins, one type of biomolecular motor, convert chemical energy of ATP into mechanical work allowing them to walk along polymer filaments called microtubules.

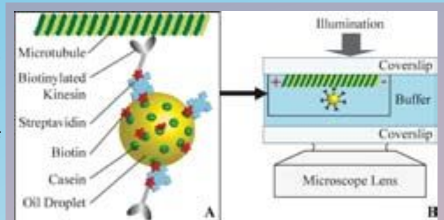

Fig. 1: (A) Schematic view of a kinesin-coated oil droplet. Biotin binds on the casein coating of the oil droplet through peptide bonds, then streptavidin attaches on biotin. Finally biotinylated kinesin binds to the streptavidin coating of the droplet. (B) Observation by DIC microscopy of the transport process

Objectives

Various types of solid particles have already been transported in vitro by kinesin molecules including microscopic beads, quantum dots or nanowires. With the long-term view of harnessing motor proteins for sensitive screening, we must prevent target molecules from interacting with each other or with the environment during the transport process. This constraint demands the development of novel types of cargo such as containers in which target molecules or particles can be placed, and suggests working with "liquid" carriers.

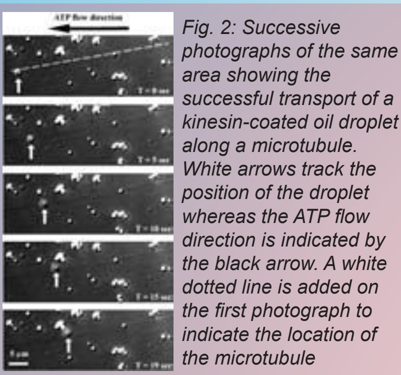

Fig. 2: Successive photographs of the same area showing the successful transport of a kinesin-coated oil droplet along a microtubule. White arrows track the position of the droplet whereas the ATP flow direction is indicated by the black arrow. A white dotted line is added on the first photograph to indicate the location of the microtubule

Results

We have selected oil droplets as cargo for kinesin motors because they are good candidates for drug delivery systems (Fig. 1). Robust and extremely versatile, they can be designed to hold volumes of materials as diverse as hydrophobic/hydrophilic molecules in extremely small quantities. We have first developed a system permitting to actively transport emulsion droplets using molecular motors (Fig. 2) and to merge them using micro-fabricated electrodes (1). A statistical study demonstrated that the diameter of the droplet displays a strong impact on the travelled distance of the kinesin motors (2). We have shown that oil droplets can be used to transport quantum dots loaded on their surface or within their core (3) and we recently proposed to harness this system for the manipulation of biomolecules.

References and Publications

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[3] Bottier C., et al., MicroTAS'09, Jeju, Korea, Nov. 2009 (poster).

Microchamber arrays for observation and characterization of in vitro translation

Contact Researcher: Dominique FOURMY, Dr.
Host Professor: T. FUJII
Keywords: single molecule, translation, ribosome, biochips

Context

The protein microarray system is a technology used for high-throughput proteomics. It simultaneously screens large numbers of protein for protein or ligand interactions, protein expression profiling, biomarker discovery, to identify the targets of biologically active small molecules, etc... [1]. Methods were recently developed that allow the synthesis of fresh protein in situ on the array using cell-free translation. However, the density of spots on the chip that can be achieved using these methods remains poor. The use of microfluidic devices containing microchamber arrays would be an advantage to allow the production of extremely dense arrays of proteins.

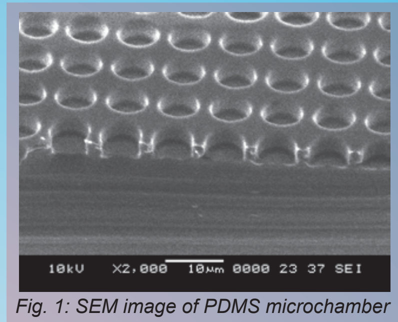

Fig. 1: SEM image of PDMS microchamber

Objectives

We propose to perform efficient in vitro protein synthesis in microchamber arrays using an in vitro translation system. This new method will allow the fabrication of high-density RNA and protein chips.

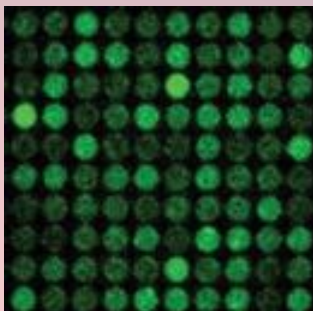

Fig. 2: Expression of GFP in microchambers from single DNA molecules

Methods

We designed microfluidic systems in order to perform highly efficient protein synthesis in microchamber arrays (Figure 1). The surface of the microchambers is first coated with a reagent to prevent protein adsorption and ensure that the internal walls of the chamber are hydrophilic for easy injection of reagents. Using a diluted solution of DNA, conditions are created such that most of the microchambers contain no more than a single DNA molecule.

Results

We demonstrate cell-free protein synthesis from single DNA molecules inside 190 fL microchambers arrayed on a chip (Figure 2). Within the microchambers, the cell-free protein synthesis proceeds via the coupled mRNA synthesis and the subsequent translation of the mRNA into proteins.

References and Publications

[1] Stoevesandt O. et. al., Expert Rev Proteomics 6, 145-57, 2009.

Liver tissue engineering: enhanced function and thick tissue formation.

Contact Researcher: Morgan HAMON, Dr.
Host Professor: Y. SAKAI .

Keywords: tissue engineering, rat fetal liver cells, PDMS, oxygen supply

Context

Knowing the mechanism of depuration process of a drug is of major interest for the pharmacokinetic researches. With the kidneys, the liver plays an important role in the depuration system. That's why tissue engineering aims to create functional organs using biomaterials and living cells. Nevertheless, the conventional in vitro conditions cannot maintain the liver cells' functions. For now, animal testing is the only trustful alternative. In order to increase the cellular activity, we focused on the enhancement of oxygen supply due to its importance in cell culture [1].

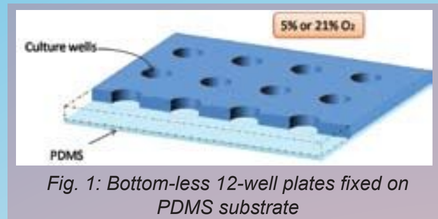

Fig. 1: Bottom-less 12-well plates fixed on PDMS substrate

Objectives

In conventional cell culture, hepatocytes (main cells of the liver) are cultured as a cell monolayer and show after 24 h a decrease of their specific functions (80 to 99% decrease) [2]. In this project, our objective is to avoid in vitro this lost of function.

Methods

We chose the fetal liver as the cells' source because it contains progenitor cells which have a highly efficient maturation capacity. Furthermore, we decided to mimic the in vivo conditions because of the crucial role of the environment [3] for in vitro specific differentiation of embryonic cells. That's why we decided to i) increase the oxygen supply to the cells, using PDMS membrane (Fig.1), ii) apply a physiological oxygen concentration (5%).

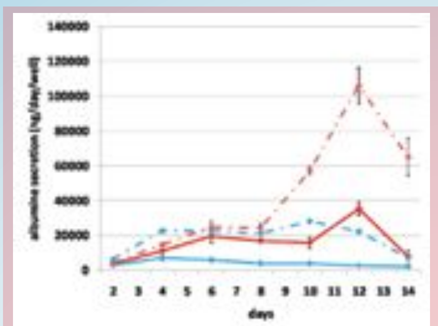

Fig. 2: Albumin secretion kinetic, measured per day and per well, for cell cultured on PDMS (dote line) or conventional culture plate (solide line) under 5% oxygen (blue) or 21% oxygen (red) atmosphere

Results

The direct oxygenation allowed us to obtain a thick tissue. This tissue was thicker when cell where cultured under 5% oxygen, whereas, the cell function was increased under 21% oxygen (Fig. 2). Those results showed the importance of oxygen supply and concentration in the cell culture becoming.

References and Publications

[1] Rotem A., Biotechnology and Bioengineering, Vol. 43, p654, 1994.
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[3] Fiegel H.C. et al., J. Cell. Mol. Med. 10, p577, 2006.

BioMEMS

Droplet generation and fusion by liquid-dielectrophoresis for biological application

Contact Researcher: Momoko KUMEMURA, Dr.

Host Professor: H. FUJITA

Keywords: droplet, microTAS, open system, enzyme reaction, MEMS tweezers

Context

The interest of biological experiments in several molecules level has been increasing. MicroTAS is suitable to treat with biomolecules, because it is possible to design and integrate channels or electrodes as fit to each experimental purpose. Also the size of these structures can be reduced to the range of several hundred nanometers to hundred micrometers. On the other hand, Fujita group has technique to trap DNA bundle with micromachined tweezers with high reproducibility [1,2]. The MEMS tweezers is also able to measure the mechanical characteristics of trapped molecules. Combining the MEMS tweezers and microTAS, a convenient biological test system will be developed for investigation of the reaction between molecules.

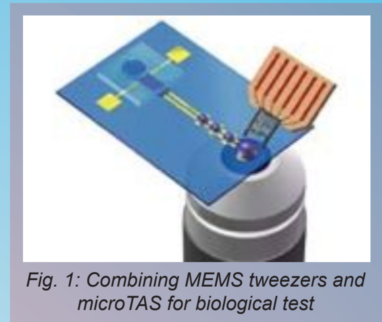

Fig. 1: Combining MEMS tweezers and microTAS for biological test

Liquid dielectrophoresis (LDEP) is one of the unique droplet generation method which is proposed T.B. Jones et al. [3]. In his paper, a droplet positioned at the end of coplanar electrodes was deformed along to all the area between electrodes by applying AC voltage. Tuning off voltage, deformed solution was separated into nano or picolitter-sized water droplets. We consider that this droplet generation method in microTAS can be applied to biological reactions with small volume samples. Additionally, as the system is in open environment, we can access to samples with external tools, such as MEMS tweezers.

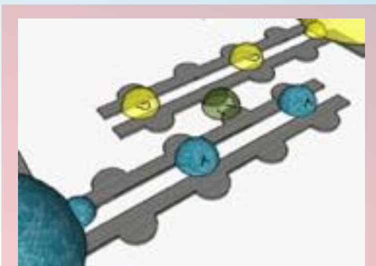

Fig. 2: Droplet generation and reaction with LDEP method which is integrated into microTAS

Objectives

This project aims to develop a microTAS for the biological application, which is able to combine with MEMS tweezers. As a first step, we concentrate the parts of micoTAS. Droplet generation and manipulation by LDEP in open environment is examined for this purpose.

Methods

A LDEP microdevice is fabricated with micromachining, such as metal deposition, photolithography, and lift-off process. With this LDEP microdevice, we conduct enzyme reaction with picolitter volume droplets.

References and Publications

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BioMEMS

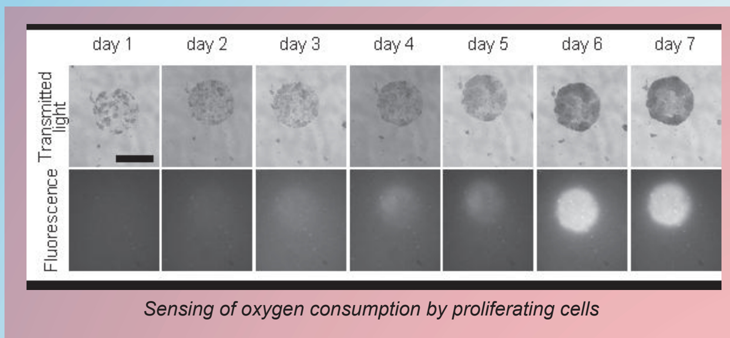
Optical sensing of cellular oxygen consumption in cell arrays

Contact Researcher: Kevin MONTAGNE, Dr.
Host Professor: Y. SAKAI
Keywords: oxygen sensing, cell culture, fluorescence

Context Micropatterned cell arrays offer many potential applications for basic cell biology, and high-throughput drug discovery. One important marker of cellular metabolism is oxygen consumption. Monitoring cellular oxygen consumption can be done by measuring oxygen concentrations in or around cells using fluorescent dyes, whose fluorescence is quenched by dissolved oxygen.

Objectives Various systems for oxygen consumption monitoring are commercially available but those only measure averaged oxygen concentration in the culture well and have poor spatial resolution. The aim is to develop a micropatterned cell array with an integrated oxygen sensor.

Methods Glass slides coated with polystyrene containing an oxygen-sensitive fluorescent dye were prepared and further coated with the gas-permeable and hydrophobic polymer polydimethylsiloxane (PDMS). Cell spotting was achieved by preparing hydrophilic patterns on the PDMS by photocatalytic lithography then seeding cells on the PDMS. Fluorescence measurements were performed by fluorescence microscopy. We speculated that by cultivating cells directly on the sensor, the fluorescence would be enhanced under the cells as cells consume oxygen.



Sensing of oxygen consumption by proliferating cells

Results By using conventional fluorescence microscopy, we can observe real-time oxygen consumption by patterned cells. Monitoring of cellular oxygen consumption can be carried out for a week and time-course and dose-response experiments with respiration inhibitors can be performed. These results demonstrate the feasibility of integrating an optical oxygen sensor in cell arrays to monitor oxygen consumption.

Multichannel planer bilayer lipid membrane chip for ion-channel analysis

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Host Professor: S. TAKEUCHI

Keywords: bilayer lipid membrane, membrane protein, microarray, nanopore

Context

A membrane protein is a protein attached to or incorporated into a biological membrane, accounting for thirty to fifty percents of all proteins. Especially transmembrane proteins, spanning the entire membrane, are considered as the target of more than half of drugs. Despite its importance, researchers on the membrane proteins have to overcome a formidable obstacle: the proteins keep their original structures and activities only at the membranes.

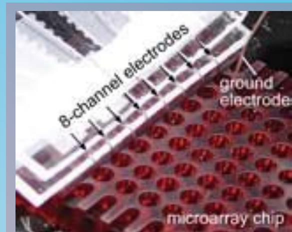

Fig. 1: Experimental setup for multichannel recordings

Objectives and Methods

We attempt to develop a multichannel BLM microarray chip with taking advantages of our previous work on the lipid bilayer microarray [1]. The microarray chip consists of three parts: (1) the intermediate polymer film suspends BLM at the micrometer-sized apertures. (2) The top part forms 96 wells as the cis-side of liquid chambers and (3) the bottom part with 12 channels, the eight top-wells in a row, as the trans-side. An eight-channel patch-clamp amplifier allows multichannel electrophysiological experiments (Fig. 1).

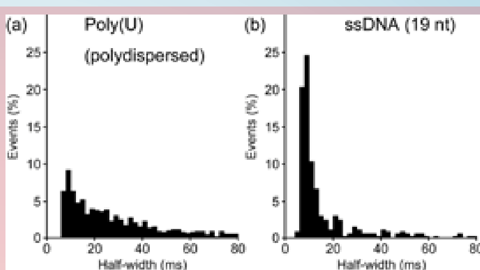

Fig. 2: Histograms of blocking peaks by translocation of different molecules

Results

The BLM was formed at the apertures by filling the top wells with buffer containing target proteins, followed by a sequential injection of lipid solution and buffer. In addition to ion-channel peptides [2] we achieved simultaneous monitoring of signals from a-hemolysin nanopore protein [3]. The system was also able to detect translocation events of nucleic acid molecules through the pore via the profile of a blocked current, promising its potential for high throughput applications (Fig. 2). We believe that this system contributes to the increase of throughput on drug screening.

References and Publications

[1] Le Pioufle B., et al. Anal. Chem., 80, 328, 2008.
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Artificial reaction networks for information processing in microfluidic devices.

Contact Researcher: Yannick RONDELEZ, Dr.

Host Professor: T. FUJII

Keywords: Isothermal amplification, unconventional computing, microchambers, reaction networks, synthetic biology

Context

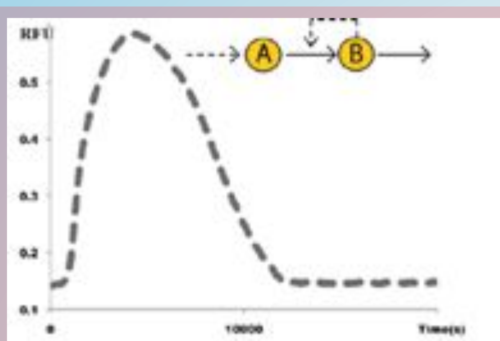
Reaction networks are of fundamental importance in biology, where they serve as the basis for information processing. However, there is currently no way to design an arbitrary chemical (artificial) reaction network *in vitro*. Finding a way to do this is the goal of this project. It will open the possibility to encode bottom-up spatiotemporal *behaviors* (as opposed to static properties) into molecular assemblies or devices.

Objectives

Build a molecular lego, and use this idea to convert microfluidic chips into functional models of neural nets, displaying computational ability.

Methods

We will combine DNA amplification reactions with microchamber technology. Thus we get both spatial and temporal control of the evolution of an observable (for example fluorescence) within the device. The first step is to demonstrate the building of simple reaction networks with interesting properties like Switches, Oscillators, or Logic gates.



The small reaction network shown in the inset has been designed to generate a fluorescent pulse.

Results

Our first results demonstrate the bottom-up design of simple functions, using DNA strands as rules to program the reactivity and observing the evolution of the molecular species through fluorescence.

Development of microdevice for high-throughput functional assay of membrane proteins

Contact Researcher: Satoko YOSHIZAWA , Dr.
Host Professor: S. TAKEUCHI

Keywords: membrane protein, *in vitro* translation, microfluidics

Context

Membrane proteins play important roles in cellular functions. Most existing assay systems of membrane proteins utilize cells harboring the target membrane protein or the membrane isolated from those cells. An abundant expression and display to the cell membrane is required which is often difficult. A general approach is awaited that is applicable to a wide variety of membrane proteins.

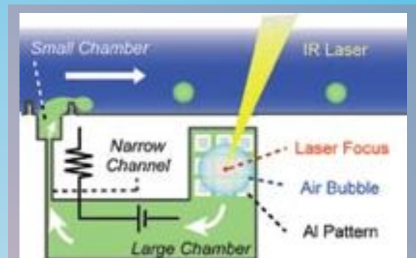


Fig. 1: Production of vesicles using a microfluidic device

Objectives

The objective is to develop a microdevice suitable for high-throughput screening of membrane protein function. Such devices may be used for a fundamental research to understand cellular response to various stimulations as well as for applications such as drug screening and highly-sensitive biosensors.

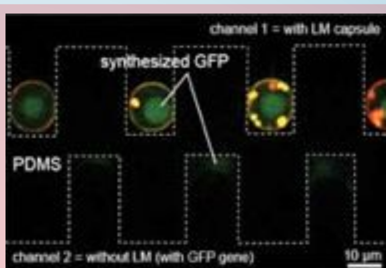


Fig. 2: GFP synthesis in a PDMS microchamber coated with phospholipid

Methods

A microfluidic device that generates monodisperse unilamellar phospholipid vesicles was constructed. Using this device, *in vitro* translational system can be encapsulated in the vesicles and GFP (green fluorescent protein) can be synthesized inside [1],[2]. We also developed a method to synthesize proteins within PDMS (poly(dimethylsiloxane)) microchambers (c.a. 15µm x 15µm x 15µm) by coating the surface of the chambers with lipids [3]. These microchambers will be further sealed with lipid bilayer. Using these systems we aim to synthesize membrane proteins and construct microdevice suitable for their functional assays.

References and Publications

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