KINESIN-BASED TRANSPORTATION AND ELECTROFUSION OF LIPID VESICLES

C. Bottier¹, M. C. Tarhan², D. Collard¹, R. Yokokawa³ and H. Fujita²

¹Laboratory for Integrated Micro Mechatronic Systems (LIMMS / CNRS-IIS), JAPAN
²CIRMM, Institute of Industrial Science (IIS), The University of Tokyo, JAPAN
³Department of Micro System Technology, Ritsumeikan University, JAPAN

ABSTRACT

A hybrid bio-electrical system for direct transportation and electrofusion of lipid vesicles is proposed; this system is the reconstruction of an intracellular transportation mechanism responsible for endocytosis. The transportation was achieved along immobilized microtubules without any liquid manipulation by using kinesin motility, a biomolecular motor. The fusion of lipid vesicles was accomplished by applying electric pulses on micro-fabricated electrodes inside a flow cell. Together with encapsulation of particles/reactants within lipid vesicles, these results give us hope to build a nano-scale reactor inspired by the cellular mechanism.

KEYWORDS: Kinesin, Lipid vesicle, Transportation, Electrofusion

INTRODUCTION

Direct transportation and electrofusion of oil droplets as carriers for hydrophobic molecules/particles were reported at MEMS '08 [1]. Here, we focused on liposomes because of their major potential to be used as hydrophilic drug carriers for drug-delivery systems [2]. Different transport modes are used to handle these carriers such as simple diffusion or active transport based on liquid flow [3].

In the present paper we propose the handling of lipid vesicles using the direct transportation method based on kinesin-microtubule system [4]. The kinesin-based transportation system combined with the electrofusion of lipid vesicles containing encapsulated molecules offers the possibility to observe the interactions between drugs/molecules in the merged liposome. Main advantage is to deal with a smaller amount of particles. For example, a liposome of 1 μm diameter contains only 300 molecules if the concentration of encapsulated solution is 1 μmol/L. Basic components of the proposed system are: i) the transportation of the kinesin-coated lipid vesicles along immobilized microtubules and ii) the electrofusion of these liposomes. In this work, the feasibility of these two main components has been demonstrated.

EXPERIMENTAL

Kinesins & Microtubules

Two types of kinesin were used. One type is full-length histidine-tagged kinesin for the immobilization of the microtubules on the substrate. The second type is biotinylated kinesin for the coating of liposomes. Tubulin was purified from porcine brains and microtubules were obtained by polymerization of tubulin. Both purification procedures of kinesin and tubulin have been previously described [1].
Preparation of lipid vesicles

Liposomes were prepared by hydrating a dry lipid film (%mol: 90% DOPC, 5% Biot-Cap-DOPE, 5% cholesterol) with an aqueous solution. Once produced, the sample was filtered resulting in a liposome size smaller than 10 μm. For the attachment between liposome and kinesin, biotinylated lipids were incorporated into the liposome during the preparation. Streptavidin-coated beads (130 nm diameter) were then used as couplers between biotinylated liposomes and biotinylated kinesins. The attachment was achieved using the streptavidin-biotin specific bond (Figure 1).

Preparation of the flow cell and motility assay

A schematic view of the liposome transportation is shown in Figure 2. First, a flow cell was built by assembling two glass slides coated with kinesins (Fig 2-a). Then microtubules (MTs) were chemically immobilized on kinesins (Fig 2-b). The last step is to add ATP to activate the kinesin-coated liposomes to move along microtubules (Fig 2-c). An inverted microscope was used for observations with an oil immersion lens. Each experiment was monitored by a differential interference contrast (DIC) setup on a microscope stage with a photometrics camera.

Electrofusion

The fabrication of the gold electrodes shown in Figure 3 has already been described [1]. The two gold electrodes were coated by an insulating layer to prevent electro-dynamic flow.

RESULTS AND DISCUSSION

In Figure 4-a, successively taken photos show the motion of liposome after addition of ATP. The average velocity was about 350 nm/s. Electrofusion, as the second part of the research, was achieved in a flow cell by applying electric pulses to two...
gold electrodes. Maintaining the pulse generation (10 V, 200 μs duration, 1 pulse/s), the fusion was observed when the liposome aggregate is close to the electrode gap (Fig 4-b).

CONCLUSIONS

We have successfully transported kinesin-coated liposomes along microtubules. Moreover, electrofusion of the liposomes using electric pulses has been demonstrated. Combined with the encapsulation of hydrophilic particles/molecules within the liposomes, both transportation and fusion of lipid vesicles imply that the system has a possibility to be integrated as a nanoscale reactor.

ACKNOWLEDGEMENTS

This work was partially supported by The Japan Society for the Promotion of Science (JSPS, fellowships #PE07503) and by The Ministry of Education, Culture, Sports, Science and Technology, Japan. Authors would like to thank to VDEC (VLSI Design and Education Center) of The University of Tokyo for the fabrication of the masks used for electrodes.

REFERENCES