FLAGELLA-DRIVEN LIPOSOMES: LIPOSOMES ACTUATED BY ATTACHED FLAGELLA

Tomoaki Kurakazu[1]*, [1]Masahiro Takinoue[1], Kaori Kuribayashi-Shigetomi[1] and Shoji Takeuchi[1,2]

[1] *IIS, The University of Tokyo, Japan*

[2] *Kanagawa Academy of Science and Technology, JAPAN*

ABSTRACT

In this study, we attached flagella to liposomes for enhancing liposome mobility (Figure 1 and 2). The flagella were detached from a biflagellate unicellular alga, *Chlamydomonas* (*Chlamydomonas oda12-lc2-bccp*), and they were then chemically bonded to the surface of the liposome. We found the mobility of liposomes with flagella was higher than that of liposomes without flagella by measuring a diffusion coefficient of the liposomes with/without flagella. Also, it was suggested that the liposomes with lower fluidity due to annexin-coating has higher mobility than non-coating one. We believe that liposomes with flagella can be utilized as high-mobility delivery vehicles to carry materials and drugs in a micrometer-sized space such as a microchip and a human body.

KEYWORDS: bioactuators, flagella, liposome

INTRODUCTION

Liposomes (figure 3A) have attracted attentions as a functional delivery vehicle that can contain biologically active compounds. If required chemicals can be infused into the vesicle and efficiently transported to local area in our body, there will be a potential of applying them to drug delivery system. To transport liposomes, biological motors are very useful. Biological motors are superior to artificial motors in that they are smaller (10 nm −10 μm) and they are more efficient at converting chemical energy into mechanical energy. Many studies have investigated the use of biological molecular motors[1-3] and living cells[4-6] as actuators in microsystems. In our prior study, we have proposed a method of attaching flagella of *Chlamydomonas* (figure 3B) to a selected region of a micro-object (made by glass or polystyrene) and advancing it [7]. Flagella can be easily detached from *Chlamydomonas* and reactivated in the presence of adenosine triphosphate (ATP, figure 3C) [8]. Here, we propose a method actuating liposomes by attaching flagella (figure 3D).

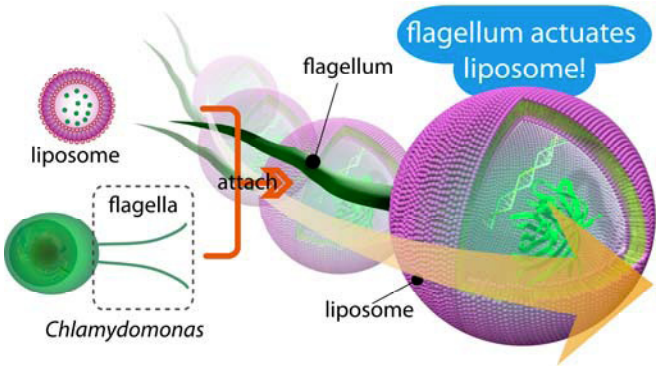

Figure 1: Conceptual illustration. Flagella detached from Chlamydomonas are attached to a liposome and actuates them.

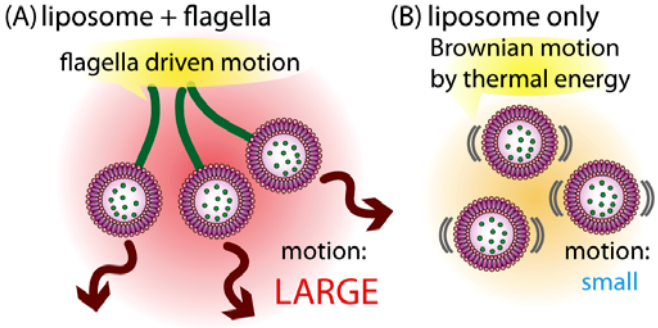

Figure 2:(A) Liposomes with flagella increase their mobility with an increase in fluctuating effect. (B) Compared with (A), liposomes without flagella move only by Brownian motion.

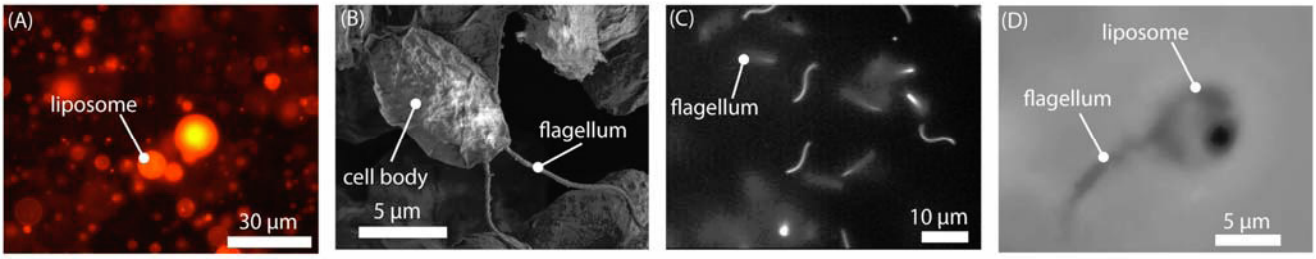

Figure 3: (A) Fluorescence image of liposomes generated by gentle hydration method. (B) SEM image of Chlamydomonas. (C) Fluorescence image of flagella detached from Chlamydomonas. (D) Optical microscopic image of liposome attached with flagellum.

EXPERIMENTAL

Figure 4 shows the method to attach flagella to liposomes. First, we cultivated *Chlamydomonas* in a liquid medium for 3days; the *Chlamydomonas oda12-lc2-bccp* has flagella tagged with a biotin by genetic engineering technique [9]. Then we collected the *Chlamydomonas* by centrifugation and added dibucaine-HCl (Wako junnyaku, Figure 4A) to induce deflagellation (flagellar shedding). We separated flagella from cell bodies by several times of centrifugation. Isolated flagella were suspended in a buffer solution and kept in ice until use. We mixed flagella with streptavidin (Sigma-Aldrich). Liposomes were made by gentle hydration method [10].The liposome consisted of two kinds of lipids, asolectin (Sigma-Aldrich) and biotynil PE (Avanti). Flagella and liposomes were strongly combined through a specific binding of streptavidin and biotin (Figure 4B). When we added ATP, flagella were reactivated.

RESULTS AND DISCUSSION

We observed the movements of liposomes actuated flagella under optical microscope. Figure 5 represents movement of liposomes with/without flagella. Sequential photographs of the movement of a liposome with a flagellum and its trajectory is shown in figure 5A and B. A liposome without flagella moved by Brownian motion (Figure 5C). By comparing figure 5B and 5C, we found that the liposome with flagella moved more widely than that without flagella moved.

Figure 6 shows mean-square displacement of liposome ($<R^2>$) with/without flagella. From the theory of Brownian motion, the dependence of $<R^2>$ on time (t) is described as $<R^2>= 4Dt$, where D is a diffusion coefficient. Figure 7 shows the diffusion coefficients calculated from Figure 6. These results suggest that the liposomes with flagella moved broader compared to the liposomes without flagella.

In order to improve the movement of liposome with flagella, we coated liposome with annexin (Annexin V, Funakoshi). Because annexin-coated liposomes have less fluidity of membrane than non-coated one [11], we investigated the dependence of the mobility of liposomes on the fluidity. As a result, it was suggested that the annexin coated liposomes has higher mobility than non-coated one.

CONCLUSION

We succeeded in enhancing the mobility of liposomes by attaching flagella to liposomes. Lower fluidity liposomes seem to have higher mobility indicates the possibility of further efficiency of transportation. We believe that this method

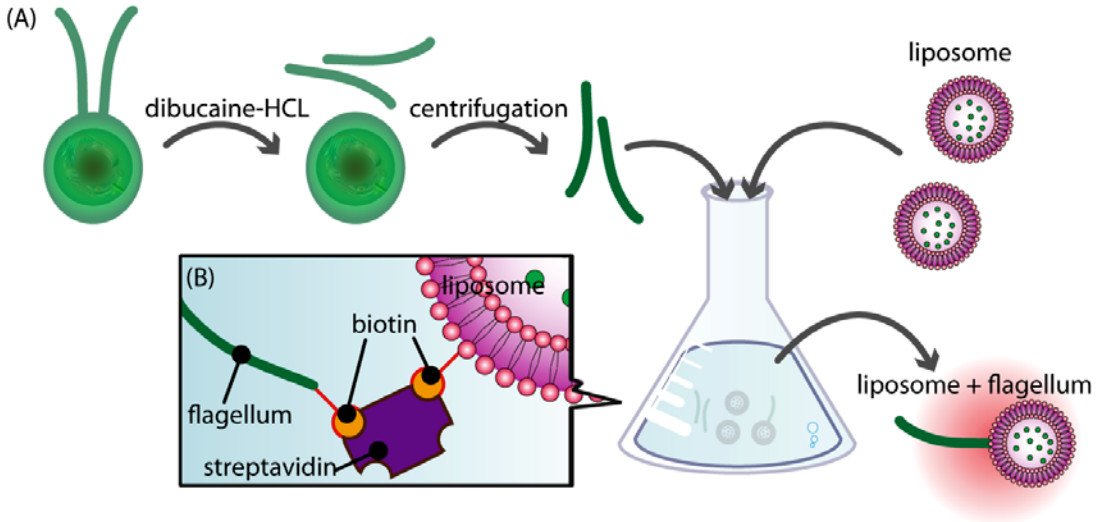

Figure 4: Attaching flagella to liposomes. (A) Flagella detached from Chlamydomonas by chemical treatment are mixed with liposomes. (B) Flagella and liposomes are combined with streptavidin, a protein, and biotin, a hydrosoluble vitamin. Compared with (A), liposomes without flagella move only by Brownian motion.

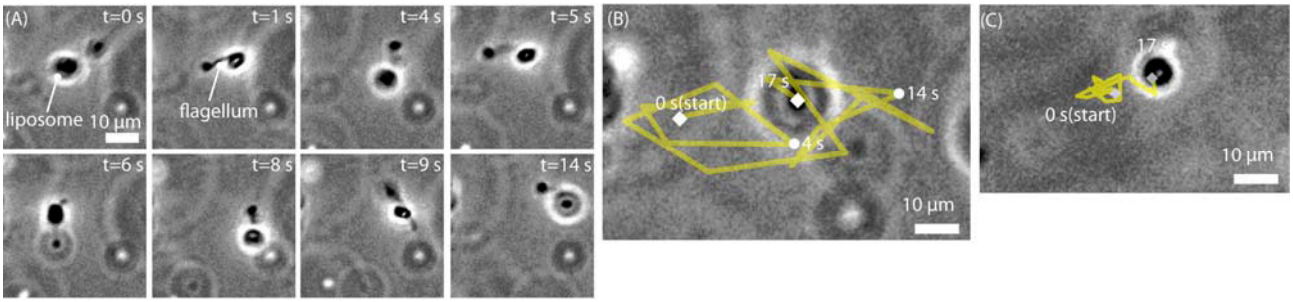

Figure 5: Movement of a liposome with/without flagella. (A) Sequential photographs of the movement of a liposome with a flagellum and (B) its trajectory (17 seconds). (C) Movement of a liposome without flagella, caused by Brownian motion. Liposomes with a flagellum moved broader area than liposomes without flagella moved.

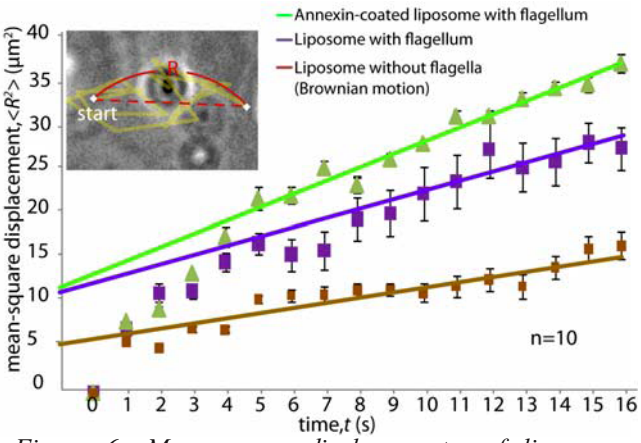

Figure 6: Mean-square displacement of liposome with/without flagella. R in the photo indicates the displacement from the starting position of the liposome. From the theory of Brownian motion, $<R^{2>}=4Dt$. D: diffusion coefficient.

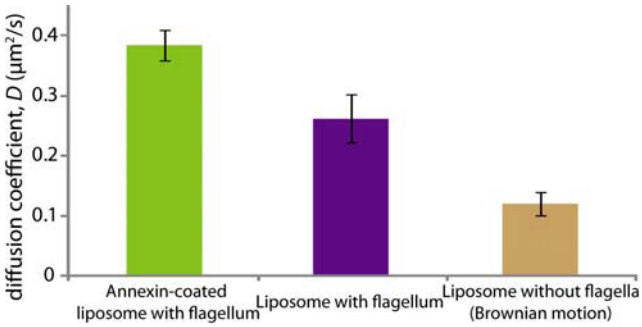

Figure 7: Diffusion coefficient calculated from figure 5. Liposomes with flagella moved broader compared to liposomes without flagella. Annexin coated liposome has less fluidity of membrane and it was suggested that liposomes with lower fluidity due to annexin coating efficiently obtained the power of flagella by comparing the mobility of the liposomes with/without annexin coating.

can be applied to high efficient bioactuating system using MEMS techniques. Since this method is simple and reproducible, it will be a powerful tool in micro-transportation systems using flagella

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CONTACT

*Tomoaki KURAKAZU, tel: +81-6452-6650; Kurakazu@iis.u-tokyo.ac.jp