Binding of Artificial Object to *Vorticella* for a Microsystem Powered by a Microorganism*

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Miniaturization, integration, and functionality of microelectromechanical systems are limited in water. One solution to this problem is to apply the micrometer-scale motors from biological systems. A suitable biological motor is a linear motor found in microorganisms such as *Vorticella*. We developed a method to attach artificial material onto *Vorticellas* through streptavidin biotin binding. We biotinylated *Vorticellas* and coupled them to streptavidin coated particles of 1, 2, 6 and 8 μm diameter. The binding force of streptavidin and biotin was strong enough to endure a high speed contraction of a *Vorticella* (several mm/s). This attachment process of artificial material is a crucial step to fabricate a microsystem powered by filaments of *Vorticellas*. [DOI: 10.1380/ejsnt.2009.673]

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I. INTRODUCTION

Microelectromechanical systems (MEMS) can sense, control, and activate mechanical processes on the micro scale and mainly work in vacuum or air. However, miniaturization, integration, and functionality of MEMS are limited in water. There are two reasons: (1) high voltages to drive MEMS require good insulation between electrodes and, (2) electronic hardware to control MEMS actuators can be easily damaged by water and must be protected; this results in the large size of the total system. These limits the sizes and functionalities of MEMS. One solution to this problem is to apply the micrometer-scale motors from biological systems. A microorganism functions effectively underwater, has an actuator and a control system in its cell body, and does not require outer system for control.

A suitable powerful biological motor is a linear motor found in microorganisms such as *Vorticella*. *Vorticella* has a filament called as stalk, which contracts and elongates in response to the concentration of calcium ion (Fig. 1). A stalk tethers to a substrate and *Vorticella* uses a stalk and finishes contraction within 9 ms. Moriyama *et al.* estimated the maximum contracting force is greater than 55.8 nN [1]. After cell membrane of a *Vorticella* is excluded, outer solution reaches inside the sheath of a stalk, and both contraction and elongation of the *Vorticella* become controllable by changing the concentration of calcium ion in solution [2]. Therefore *Vorticella* can be used as a linear actuator equipped with a sensor for calcium ion. In order to obtain an effective work, it is indispensable to attach an artificial microobject onto its surface and as-

FIG. 1: A *vorticella* contracts and elongates in response to concentration of calcium ion.

semble a mechanical system. However, the method to bind artificial material to *Vorticella* has not established yet.

Some methods to bind cells and artificial materials have been reported [3–6]. Since the surface conditions of cells are diverse, attaching methods differs from one case to another. Weibel *et al.* synthesized peptides with a high affinity for *Chlamydomonas reinhardtii* and attached the particles onto the cell body [3]. Darnton *et al.* and Steager *et al.* coupled microsize objects to *Serratia marcescens* through affinities of PDMS (Polydimethylsiloxane) [4] and SU-8 [5], respectively. Hiratsuka *et al.* anchored microobjects to *Mycoplasma mobile* [6]. They used the property that streptavidin has a strong affinity for a vitamin, biotin. They attached biotin to amino groups on the surface of the cells (this attachment process is called biotinylation) and microobjects were coated with streptavidin. They mixed the objects and the cells, and coupled the microobjects to the cells. As described before, in order to bind artificial material to a cell, it is essential to understand the surface condition of a cell and use a material with an affinity for the cells surface.

We developed a process to attach a microobject onto a *Vorticella* through an adhesive property that streptavidin

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FIG. 2: A schematic diagram to bind artificial material onto a *Vorticella*. (a) A *Vorticella* has amino groups on its surface. (b) Biotinylation of *Vorticella*. Biotin-PEO₁₂-NHS which has amino-reactive group is chemically linked to the *Vorticella*. (c) Then streptavidin coated particle attaches the *Vorticella* through streptavidin-biotin binding. (d) The magnified view of the region enclosed by the box in (c).

binds biotin as a first step to realize microsystems driven by harnessed microorganisms. Amino groups existing on the surface of a *Vorticella* were modified and coated with biotin. Then we introduced streptavidin-coated particles near the *Vorticella* and attached the particles. We optimized the composition of a buffer solution for *Vorticellas* to keep them alive and active over two hours.

II. EXPERIMENTAL

Vorticella Picta was collected from lake Hattyou in Yoshimi, Saitama. We isolated *Vorticella Picta* and cultivated them in a culture medium containing bacteria at room temperature, around 25°C. We kept 18×18 mm coverslips floating (Matsunami Glass Ind., Ltd., thickness: 0.12-0.17 mm) on the culture medium for a few days until a suitable number of *Vorticellas* attached onto the coverslips. We made a filtered infusion of dry yeast, EBIOS (Asahi Food & Healthcare Ltd., Tokyo, Japan) as shown below and used it for the culture medium. The medium was infused by autoclaving 2.5 g/l of EBIOS in Milli-Q water at 120°C for 20 minutes. Then the infused liquid was filtered through a 0.2-μm filter unit (NALGENE® Disposable Filter Unit, 566-0020; Capacity: 500 mL) to remove solid materials. Prior to use, we diluted this filtered liquid tenfold with Milli-Q water and used as the culture medium.

We assembled a flow chamber and conducted all steps of chemical reaction of *Vorticellas* and attachment of particle in the chamber. The flow chamber was composed of three parts. (1) *Vorticella*-attached coverslip, as described above. This part was used as an upper side of the flow chamber. (2) A coverslip of larger size than the upper side (24×36 mm, Matsunami Glass Ind., Ltd., thickness 0.12-0.17 mm). This part was used for the bottom side of the flow chamber. (3) A spacer, which is sand-

FIG. 3: Optical micrographs of *Vorticellas* which particles attached through streptavidin-biotin binding. The diameters of each particles are (a) 1 μm, (b) 2 μm, (c) 6 μm, respectively. Red arrows indicate attached particles. Scale bars: 50 μm.

wiched between two coverslips not to crush *Vorticellas*. We coated a paper with silicone grease (DOW CORNING TORAY HIGH VACUUM GREASE, Dow Corning Toray Co., Ltd.) to make it adhesive. We pipetted different types of solutions from one side of the fabricated flow chamber and removed it from the other side by capillary action using Whatman #2 filter paper.

We injected Dryl's solution (2 mM Na₃Citrate·2H₂O, 0.6 mM NaH₂PO₄, 1.4 mM Na₂HPO₄, 1.5 mM CaCl₂·2H₂O) into the chamber and washed *Vorticellas*. Then we introduced 0.4 mM NHS-PEO₁₂-Biotin (Pierce,

FIG. 4: Time-series optical micrographs of 8 μm particle in diameter, which attached to a *Vorticella* during a contraction and elongation. The location of the particle is almost the same (a) before contraction and (e) after elongation. Scale bar: 20 μm .

EZ-Link® NHS-PEO₁₂-Biotin, 21312) in Dryl's solution to the chamber and coated biotin tags with *Vorticellas* from the amino groups on the surfaces of *Vorticellas* as shown in Fig. 2. We kept *Vorticellas* in the solution for over 30 min. Here, we prepared two types of samples, biotinylated and non-biotinylated samples in order to examine the effect of biotinylation. After the biotinylation, we injected Coupling Buffer (0.1-0.2% Bovine Serum Albumin [Sigma], KCl 50 mM, MgCl₂ 2 mM, MOPS-KOH 10 mM) to remove the unreacted solution.

We used streptavidin coated particles of four different sizes from two suppliers. Particles of 1, 2 and 6 μm in diameter were purchased from Polysciences, Inc. (streptavidin conjugated microspheres, 24162, 24160 and 24158 respectively). Particles of 8 μm diameter were obtained from micromod Partikeltechnologie GmbH (micromer® 01-19-803). Prior to use, we washed the particles with the coupling buffer by centrifuging the particles and discarding the supernatant several times. We adjusted the final concentration of the particles to 0.137, 0.063, 0.069, 0.241% (v/v) for the particles of 1, 2, 6 and 8 μm in diameter, respectively.

After we injected the particles into the chamber and mixed them with *Vorticellas*, we put the flow chamber on an inverted microscope (IX71, Olympus) equipped with an objective lens (Magnification 60 $\times$, N.A. = 0.7, Olympus), and a CCD camera (Resolution 512 $\times$ 512 pixel, 16-bit, Cascade II 512, Photometrics). We evaluated attachment of the particles to the movements of the particles. When particles follow the motion of *Vorticellas*, we regard these particles are attached.

III. RESULTS AND DISCUSSIONS

We describe a comparison between biotinylated sample and non-biotinylated one in order to measure the effect of biotinylation. In case of biotinylated sample, the particles followed movement of cilia or stalks of *Vorticellas*. Meanwhile, in case of non-biotinylated sample, we did not observe the particles follow the motions of *Vorticellas*. The particles moved randomly in the solution. The particles did not attach onto the surfaces of *Vorticellas*. This comparison between two samples reveals that the

biotinylation helps to attach streptavidin particles.

The particles of 1, 2, 6 and 8 μm diameter attached *Vorticellas* and followed their motion (Figs. 3 and 4). Number of attached particles depended on the size of the particle. As the size of the particles decreased, the number of attached particle increased. *Vorticellas* were alive, and contracted and elongated over two hours in our optimized buffer solution.

Particles of 1 μm diameter attached *Vorticellas* more than ones of other diameters. The particles attached to (1) cilia, (2) cell body and (3) stalk in decreasing order. Most of the *Vorticellas* had over 20 particles attached. Figure 3(a) shows many particles attach to cilia of a *Vorticella* and the cilia are tied up. At most 6 particles attached to a single cell body and 3 particles were coupled to a single stalk. Numbers of attached particles depended on the lengths of stalks. There was a tendency that the longer stalk had more attached particles. Since many particles attached the glass surface near the anchoring points, it was hard to observe anchoring points.

Figure 3(b) shows the particles of 2 μm diameter coupled to cilia. The particles in the figure followed the motion of the cilia. Number of particles attached to cilia is fewer than half the number of the samples of 1 μm in diameter. Some particles attached to cell bodies. Almost all particles were floating during observation.

A particle of 6 μm diameter attached to cilia as shown in Fig. 3(c). The particle moved according to the cilia motion. Number of particles was less than the cases of particles of 1 and 2 μm in diameter. Cilia had two particles at most. We did not observe particles which attached stalk. Almost all particles sank down to the bottom and number of the attached particles was less than the number of particles of 1 and 2 μm in diameter.

A particle of 8 μm in diameter attached onto a *Vorticella* (Fig. 4). The particle (indicated by red arrows) followed the motion of the *Vorticella* while it contracted and elongated. In the figure, all particles except the attached one were not floating and stayed on the bottom side of the coverslip. The binding force of streptavidin and biotin was strong enough to endure the high speed contraction of a *Vorticella* (several mm/s). Trace of the attached particle during the elongation is depicted in Fig. 5. Number of particles was less than other particles. We did not ob-

FIG. 5: Time-series trace of the particle which attached to the *Vorticella* in figure 4 during elongation. Numbers near red points in the view area represent time, t [s], from starting point of elongation. At $t = 0$ s, the *Vorticella* fully contracted. From $t = 0$ s to $t = 6$ s, the *Vorticella* gradually elongated rotating the cell body. When $t = 6$ s, the *Vorticella* finished its elongation.

serve particles which attached stalk. Almost all particles were deposited on the coverslip.

The distribution of the particles is explained by contact probability between particle and each region of a *Vorticella*. Cilia move constantly in the solution generating the flow at the speed of above $2500 \mu\text{m/s}$ [7] and the contact probability is the greatest. On the other hands, cell body and stalk move less than the cilia and the contact probability is smaller than one of the cilia.

There are two reasons that larger particle is more difficult to attach than small one: (1) Small diameter particles have higher surface area to volume ratio and are easier to attach to *Vorticellas* than larger particles. (2) The larger particles are more easily precipitated than the smaller particles. When a particle is large, there will be less floating particles. Presuming that we use the same density of par-

ticles and we increase the size of particles, sedimentation velocity, which is proportional to the square of diameter increases as shown in Eq. (1). Therefore the larger particles are more easily precipitated than the smaller particles.

$$v = \frac{d^2 (\rho - \rho_0) g}{18\eta}, \quad (1)$$

where d : diameter of particle, ρ : density of particle, ρ_0 : density of solution, g : gravity acceleration, η : viscosity of solution.

It is preferable that artificial material attach to the cytoskeletal structure rather than to fluid molecules inside a cell membrane. This is because the cytoskeletal structure can transmit the contracting and elongating force of *Vorticella* to attached material more easily than the fluid-like cytoplasm. Therefore, material attached to the cytoskeletal structure can follow the motion of the *Vorticella* more easily. It is presumed that larger particles need stronger binding forces to stay attached because of the greater viscous force associated with larger particles.

IV. CONCLUSIONS

We developed a method to attach an artificial material to microorganisms through streptavidin-biotin binding. We biotinylated *Vorticellas* and coupled streptavidin coated particles of 1, 2, 6, 8 μm diameter to them. This method enables us to assemble a device powered by *Vorticella*. This attachment process of artificial material is a crucial step to fabricate a microsystem powered by the stalks of *Vorticellas*.

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- [1] Y. Moriyama, S. Hiyama, and H. Asai, *Biophys. J.* **74**, 487 (1998).
 - [2] W. B. Amos, *Nature* **229**, 127 (1971).
 - [3] D. B. Weibel, P. Garstecki, D. Ryan, W. R. Diluzio, M. Mayer, J. E. Seto, and G. M. Whitesides, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11963 (2005).
 - [4] N. Darnton, L. Turner, K. Breuer, and H. C. Berg, *Biophys. J.* **86**, 1863 (2004).
 - [5] E. Steager, C.-B. Kim, J. Patel, S. Bith, C. Naik, L. Reber, and M. J. Kim, *Appl. Phys. Lett.* **90**, 263901 (2007).
 - [6] Y. Hiratsuka, M. Miyata, T. Tada, and T. Q. Uyeda, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 13618 (2006).
 - [7] M. A. Sleight and D. Barlow, *Trans. Am. Microscopical Soc.* **95**, 482 (1976).