LIGHT CONTROLLED INTEGRATABLE SINGLE CELL MICRO ROTARY VANE PUMP

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ABSTRACT

This paper reports a light controllable single cell driven micro rotary vane pump for transport fluid in small space based on a new type of bio-actuator: *Euglena*. Different from other types of bio-actuators, *Euglena* has features of both animals and plants, which will allow, for the first time, bio-actuator functioned in fresh and salt water and take nourishment through photosynthesis. To demonstrate the potential of *Euglena* actuator, we fabricated a simple chamber as a pump structure, and let the *Euglena* swim through the chamber. Then, by irradiating the *Euglena* with light in a range from 400 to 420 nm, the *Euglena* started a fixed location-rotation, which generated a flow. The particles flow by the generated flow was confirmed.

INTRODUCTION

A bio-actuator is a kind of actuator with biological organism integrated. Because advantages of self-actuated, wireless, and mechanochemical transducers that require no externally coupled energy source or stimulate, researchers of micro devices and cellular mechanical that using bio-actuators has become popular[1][2]. In most of the previous research, mainly two kinds of cells and tissues were used demonstrate the potential of bio-actuator, which are those of mammalian[3]–[6] and insect[7], [8]. However, these mammalian muscle cells and tissue-integrated devices require precise environmental control to keep the contractile ability of the muscle cells. The culture medium must be often replaced; pH and temperature of the medium should be strictly controlled around 7.4 and 37°C, respectively.

On the other hand, although the tissues of insects are generally robust over a much wider range of living conditions comparing with those of mammals, they are still required environments control and it was hard to be controlled indirectly without a chemically regent. Single cell size actuator is also hard to fabricate.

Recently, our group reported the micropump fabricated by earthworm muscle[9]. Compared with above bio-actuator, the natural muscle of earthworms is an excellent actuator to drive fluids due to its membranous structure, strong force, short response time, and controllability. However, it is big (cm order), heavy, and requires electronic control, so it still could not be used to fabricate small and simple controllable actuator.

The bio-actuator directly using an original single biological cell, capable of functioning in fresh and salt water, and take nourishment through photosynthesis, easily controlled by the light source was never reported. To satisfy all the above requirements, we focused on a single cell size, a self-driven creature named *Euglena*. *Euglena* is quite special species because it has photosynthesizing chloroplasts within the body of the cell, which enables them to feed by autotrophy, like plants. In addition, phototaxis of *Euglena*s is controlled by a primitive visual system consisting of a photoreceptor, at the base of the flagellum, and a pigmented shading device called an eyespot, these makes it sensitive to light [10], and capable of moving toward the light. In other words**,** by control, the on/off action of light, the motion of the *Euglena* is also possible.

In this paper, different from previous work, we first found that if we irradiated the *Euglena* with light that wavelength in a range from 400 to 420 nm, *Euglena* started a fixed location self-rotation; the rotation was fixed location, stable and relatively fast. By using this phenomenon, we are possible to trap, hold and rotate the *Euglena* in a designed structure. Since the pump is a most popular component for integrated microfluidic devices, therefore We select a rotary vane pump structure to demonstrate the potential of *Euglena* as a new type of bio-actuator (Fig.1).

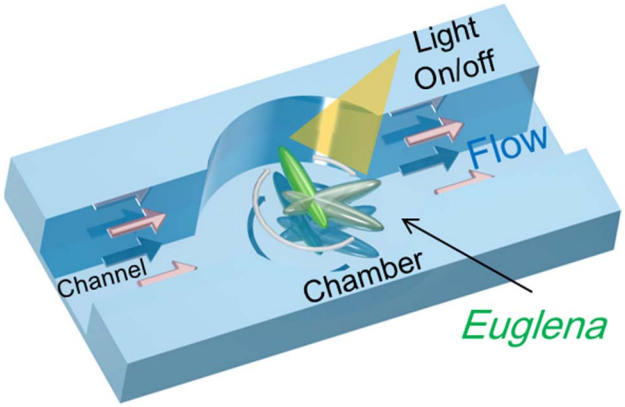

Figure 1: Concept image of the micro rotary vane pump. This pump is a positive-displacement pump that consists of only Euglena that rotates inside of a PDMS made circuit chamber and controlled by only light.

MATERIAL AND METHOD

Material

Euglena (euglena Co.,Ltd.,Tokyo, Japan) were grown in culture conditions and collected for large-scale purification. The average size of *Euglena* we used was in a range from approximately 50 – 150 μm. Because the different size of *Euglena* leads to a different characteristic of rotation, we first investigate the relation between rotation speed and size of *Euglena* to design the pump with proper dimension. The initial concentration of *Euglena* used in our experiment was 1×10^3 /ml.

Design of Prototype

We designed a micro rotary vane pump based on the structure as shown in Fig.1. The pump consisted of a

channel and a chamber. The channel was used to introduce *Euglena* and direct the generated flow. The chamber was used to offer a place where *Euglena* rotated in. In Fig.2A, the dimension of the channel (10 mm × 100 μm) and the chamber (diameter of 200μm) structure at a position that 5.85 mm from inlet orifice was given.

Fabrication of Prototype

We used Polydimethylsiloxane (PDMS) to fabricate porotype of the pump by a general photolithography process (Fig.2B). The single pump was a two-layer bonded structure. We first convert the design of the pump to a photomask. Then we spin coated SU-8 register layer on a glass slide, after prebake processes, we exposure the structure to SU-8 register layer by using the photo mask. After that, we developed the SU-8 layer. After post bake, we replicated the structure from SU-8 to PDMS. Finally, we peeled off the PDMS layer and bonded it to slide glass to close the porotype by plasma assisted bonding process. Inlet and outlet were punched on the PDMS.

Experiment Setting

To set up our prototype pump device, we used a syringe pump (Fusion 200; Chemyx, Stafford, TX, USA). Micro-tracking particles were used to visualize the fluid flow. Fluorescent spherical polystyrene particles (FluoroSpheres; Molecular Probes, Invitrogen, Carlsbad, CA, USA) with diameters of 2, and 10 μm were dispersed in the fluid (1000×, and 1000× with distilled water, respectively). The channel was observed using an optical zoom microscope (EMZ-C 0.5-4X; Kyowa Optical, Nagano, Japan), with a 2.5× extender (EMZ; Kyowa Optical). All of the experiments were carried out at room temperature.

Experiment procedure

We first introduce the *Euglena* to the channel of the pump and flow them toward to the chamber. After introducing the solution contained *Euglena*, the syringe , and connections between syringe and pump device were released to avoid the possible influence.

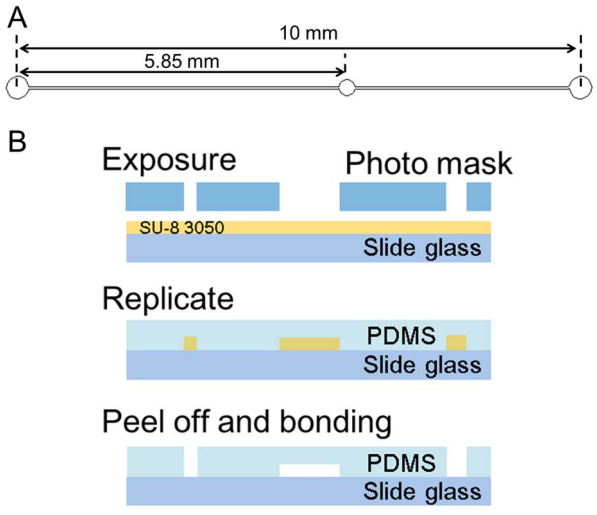

Figure 2: Design and prototype of the micro rotary vane pump. (A) The dimension of the porotype. (B) The brief fabricating processes including exposure the structure to SU-8 register (SU-8 3050) by using photo mask, development of SU-8, replicate the structure from SU-8 to PDMS, peel off, and bonded the PDMS to a sliding glass to closed the porotype, inlet and outlet were punch on the PDMS.

When the *Euglena* arrived the target chamber, we start the irradiation of the light. Then the *Euglena* started to rotate in a fixed location and has the pump functioned, generates the flow. To verify the generated flow, different types of particles (diameter of 2 μm and 10 μm) was also introduced to the porotype pump with *Euglena*. The initial concentration of them was also calculated as 1×10^3 /ml.

RESULT AND DISCUSSION

The relation between rotation speed and size of Euglena

In this investigation, we found that motion of the *Euglena* was immediately switched a random linear motion to a fixed location rotation when we started the light irradiation (Fig.3A and B).

The Fig.3 showed the investigating result of the relation between rotation speed and size of *Euglena*. The rotation speed was influenced by the size of *Euglena*, smaller *one* rotates faster but the flow rate was considered lower than a larger one. The reason we considered was larger size ones has more surface area that leads to more drag force to reduce the speed of the motion. On the other hand, smaller one rotated fast but not generated flow effectively due to less surface area. Therefore, in the demonstration, we put the generation of flow rate as a top priority, and we selected to use an approximate 100-μm diameter scale *Euglena.*

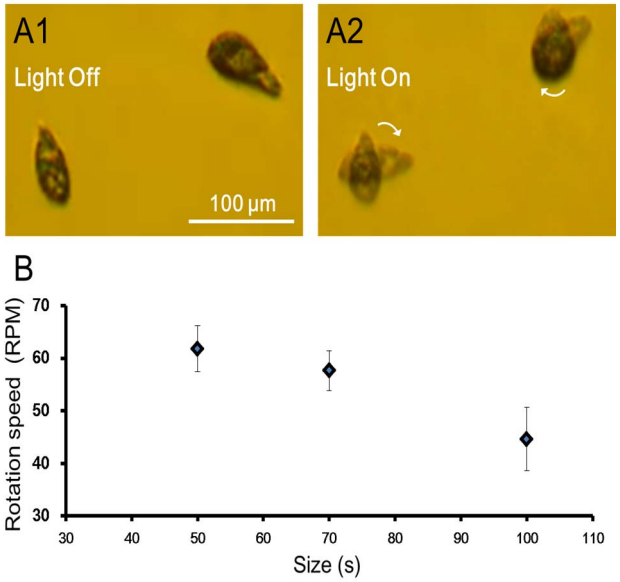

Figure 3: Light controlled rotation of Euglena. (A1)When the light turned off, Euglena had a random motion. (A2) Overlay image of the moment of light turned off and on, Euglena started a fixed location self-rotation. The scale bar indicted 100 μm. (B) The rotation speed of Euglena in different size was different, smaller one rotated faster than larger ones.

Prototype Rotary Vane Pump

Fig.4A shown the porotype of the micro pump. These were twelve micro pumps on a block of the PDMS. Fig.1B showed the detail of the center area of a single pump unit. The structure was clear and no leakage. In Fig.3C, the chamber was also clear and the diameter was approximately 200 μm as designed.

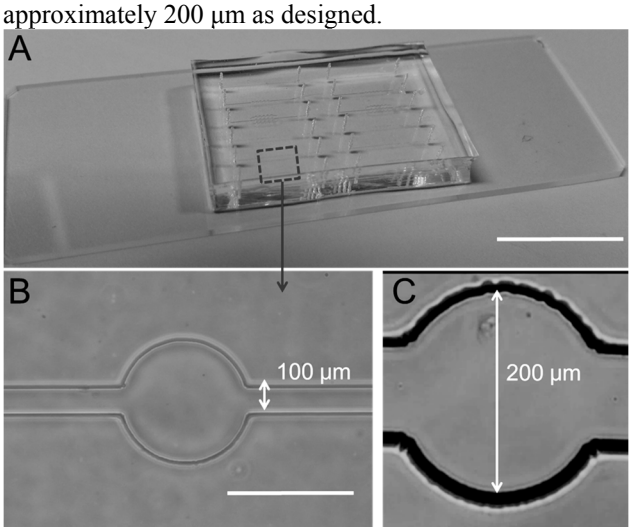

Figure 4: Prototypes of the rotary vane pump device. (A) The total porotype device including twelve single pump units. The scale bar indicted 1200 μm. (B)The detail view of the center area of the single pump unit. The scale bar indicted 200 μm. (C) The enlarge view of the chamber used in this paper. The size of the chamber was 200 μm.

Capture of *Euglena*

The image of experiment process was shown in Fig.5A (1-3). After introducing *Euglena*, they started a randomly swim through channels and chambers. We monitoring some of them, and when they arrived the chamber filled with several types of the micro polystyrene particle (diameter of 2, 10 μm) , then we started to irradiate, the *Euglena* then started to shrink a little, then start the rotation.

Rotation of the *Euglena*

The images of the *Euglena* rotation were shown in Fig.5B1-4. The rotation speed of *Euglena* was about 50 rpm. Although the position of rotation was near to one side of the chamber, the generated flow was confirmed. The rotation was observed as a stable and clockwise motion. During the rotation of *Euglena*, we measured the position of these particles, to measure the flow generation.

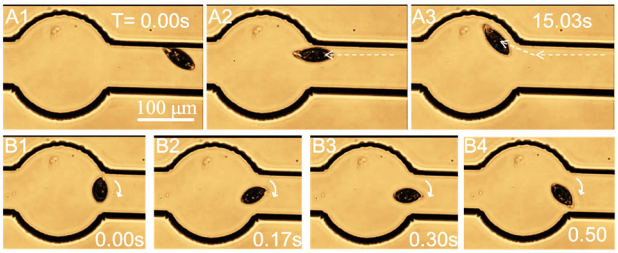

Figure 5: Images of experiment process of capturing and rotating of a Euglena. A (1-3) When the Euglena randomly swam through the chamber which was filled with several types of microparticle (diameter of 2, and 10 μm). B(1-4) The irradiation started and the Euglena began a fixed location rotation. The speed of rotation was measured by image, and the flow generation could be confirmed by displacement of particles.

Flow generation of the rotary vane pump

As shown in Fig.6A (1-4), the motions of particle induced by generated flow were confirmed and analyzed by image process (ImageJ). We totally tracked 4 particles in the flow. The displacements of particle number from 1 to 4 (Fig.6A) were shown in Fig.6B, clearly increased during the rotating period of the pump. Furthermore, number 5 was the tracking point of the tail of *Euglena,* clearly indicated that *Euglena* had a stable fixed location rotation and the rotation speed was approximately 50 rpm. After the irradiation, the *Euglena* started the random motion again.

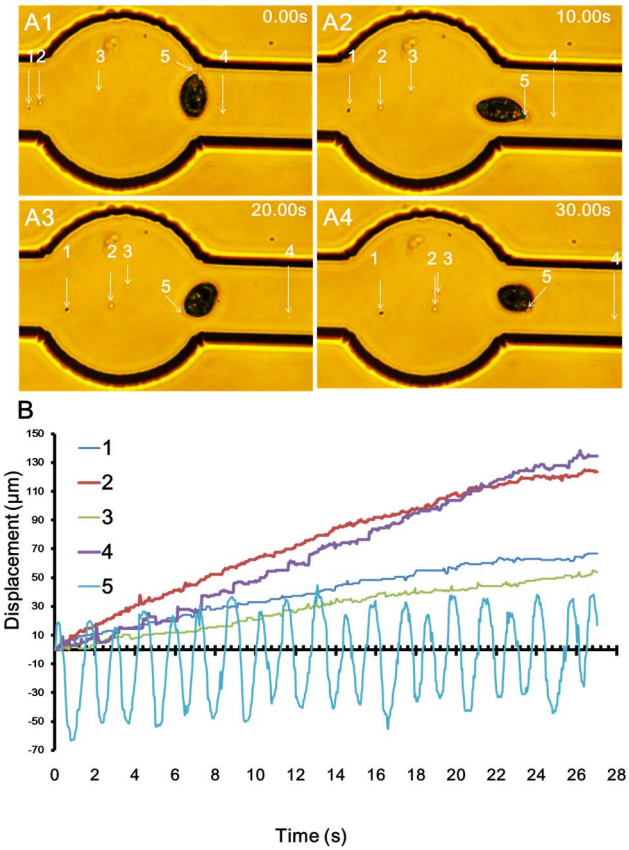

Figure 6: Flow generation confirmation. Several motions of particle induced by flow generated by the pump were confirmed by an image process. The displacements (analyzed by ImageJ) of particle number 1 to 4 (B) clearly increased during the rotating period of Euglena. Furthermore, number 5 was the tracking point of the tail of Euglena clearly indicated that Euglena had a steadily fixed location rotation and the rotation speed was approximately 50 rpm.

Based on the displacements of the particle we calculated the flow rates by using the follow equation.

$$F = W \times D \times \frac{L}{T} \qquad (1)$$

where F is estimated flow rate generated by the pump.

W was the width of the channel. D was the depth of the channel.

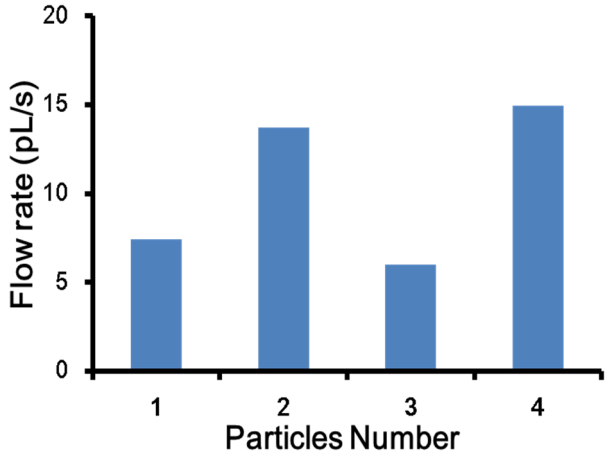

Figure 7: Calculated flow rate based on the displacement of the particles.

The maximum generated flow rate was calculated from 6 to 15 pL/s which was 8 times larger than previous work [5] and only 1/20 in size.

Overall, the *Euglena* could be used as a controllable bio-actuator for integrated into small devices. The total size of the pump could be reduced to 100 μm for effectively generate flow. Compare with other small size bio-actuators, *Euglena* has more robustness.

CONCLUSION

In this paper, we reported a simple, small, single cell driven, light controllable micro rotary vane pump based on a new type of bio-actuator: *Euglena*. To demonstrate the potential of *Euglena* actuator, we first investigated the relation between rotation speed and size of *Euglena.* And, based on the proper size of *Euglena,* we design and fabricated a simple device contained a channel and a chamber as a pump. Then we let the *Euglena* swim through the chamber, captured it by irradiating the *Euglena* with light in a range from 400 to 420 nm. Finally, the *Euglena* started a fixed location-rotation, which generated a steady flow. The flow was confirmed by the displacements of particles. The function of *Euglena* pump was confirmed.

ACKNOWLEDGEMENTS

This research was funded by the ImPACT Program of the Council for Science, Technology and Innovation (Cabinet Office, Government of Japan).

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