Long Term and Room Temperature Operable Muscle-Powered Microrobot by Insect Muscle

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Abstract. This paper describes an insect muscle-powered autonomous microrobot (iPAM) which can work long-term at room temperature without any maintenance. The iPAM consisting of a DV tissue and a frame was designed on the basis of a finite element method simulation and fabricated. The iPAM moved autonomously using spontaneous contractions of a whole insect dorsal vessel (DV) and the moving velocity was accelerated temporally by adding insect hormone. These results suggest that the insect DV has a higher potential for being a biological microactuator than other biological cell-based materials. Insect dorsal vessel (DV) tissue seems well suited for chemically regulatable microactuators due to its environmental robustness and low maintenance.

Keywords: Microrobot, Bioactuator, Chemical stimulation, Insect, Dorsal vessel, Neuroactive chemical.

1 Introduction

Recently, mammalian muscle cells have received considerable attention as a novel actuator for microdevices [1]-[7], and reported bio-hybrid microdevices using mammalian heart or skeletal muscle cells include a pillar actuator [1][2] and a micro heart pump [3][4]. Muscle cells are well suited to work in a microspace due to their size and their high energy-conversion efficiency. For instance, muscle tissues and cells are soft and small, and they can contract using only the chemical energy in adenosine triphosphate (ATP). However, these devices require precise environmental

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control to keep the contractile ability of the muscle cells. The medium must be replaced every few days and pH and temperature must be kept around 7.4 and 37 °C, respectively.

Tissues and cells of insects are generally robust over a much wider range of living conditions as compared to those of mammals. As an example, the characteristics of insect dorsal vessel (DV) tissue and rat cardiomyocyte (CM) are summarized in Table 1 based on literature data; values for the DV tissue were obtained from [8], [9], and [10] and the values for the rat CM tissue were obtained from [1] and [7]. We previously proposed to utilize insect DV tissue and cells as an actuator and we demonstrated a micropillar actuator which worked at room temperature for more than 90 days without medium replacement [8]. Surprisingly, the micropillar actuator could work at temperatures from 5 to 40 °C though the contracting velocity and frequency of the micropillar decreased with lowering of temperature and the actuator was irreversibly damaged at 40 °C [9].

There are several advantages when utilizing biological tissue and cells as an actuator. One of them is chemical controllability of the contractions. It has been reported that epinephrine, acetylcholine, and caffeine, which are physiologically active chemicals in vertebrates, have an effect on heart beat of Periplaneta americana [11]. On the other hand, crustacean cardioactive peptide (CCAP) has been found in the moth Manduca sexta [12]. We have already confirmed that CCAP has an ability to accelerate the heart beat of an inchworm in vitro [13].

In this paper, we demonstrate a pantograph-shaped microrobot (PSMR) as an example of an insect muscle-powered autonomous microrobot (iPAM). The PSMR using a whole DV will work autonomously at room temperature without any maintenance for a long time. At first, the PSMR is designed and its deformation by the contraction of the DV tissue is simulated using finite element analysis simulation software. Then, the frame of the PSMR is fabricated by molding. After that, the PSMR is fabricated by assembling the whole DV onto the frame. The PSMR is evaluated by measuring the moving distance from the side. Finally, we attempt to accelerate the PSMR movement by adding the neuroactive chemical, CCAP.

Table 1. Comparison of insect DV tissue and rat CM

	DV tissue	Rat CM
Lifetime	90 days	14 days
Contractile Frequency	0.2 Hz	1 Hz
Contractile Force	96 μN	3.5 μN
Medium Replacement	Not Needed	2 to 3 days
Viable Temperature	5 to 35 °C	37 °C

2 Design of PSMR

We designed the PSMR as shown in Figure 1. The contractile force of a whole DV has been reported to be 96 μN [10]. Based on this value, we designed a frame

consisting of a pantograph-shaped body and four legs. The frame was made of polydimethylsiloxane (PDMS) and its width and height were each 200 μm. Two slits were made at each end of the body so as to hold the DV tissue in place when it was wrapped around the body. The diameter of each leg was 200 μm. The front legs were 1100 μm long and the rear legs were 600 μm long.

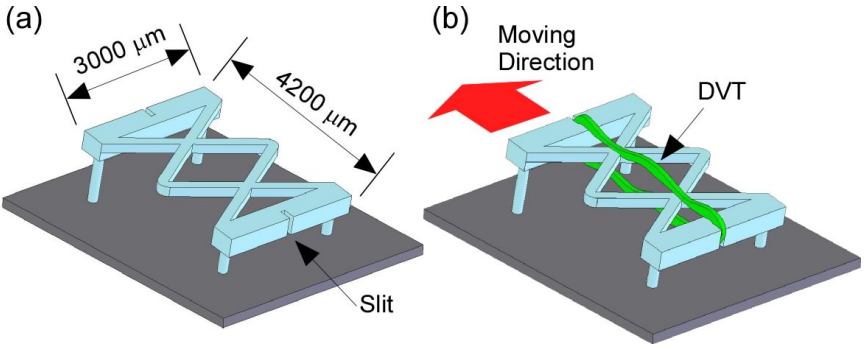

Fig. 1. Respective illustrations of PSMR (a) before and (b) after DVT assembly. The arrow in (b) shows the moving direction of the PSMR.

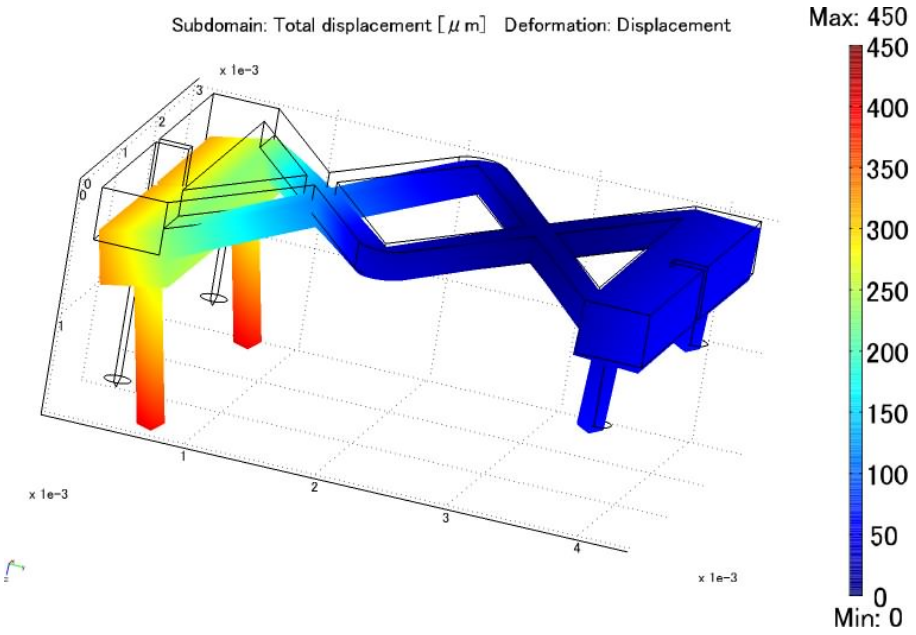

Fig. 2. Simulation results of deformation of PSMR. Young's modulus and Poisson's ratio of PDMS were set to 1.8 MPa [14] and 0.48, respectively.

Next, we calculated the deformation of the PSMR by the DV tissue contraction using the simulation software COMSOL Multiphysics. In this simulation, a linear elastic model was used. The contractile force was loaded onto the edges of the slits. The analysis results are shown in Figure 2. The maximum displacement of the front leg was about 420 μm. The distance between the front leg and the rear leg was reduced by 420 μm, which was more than the diameter of the leg. This result strongly suggests that the PSMR will move by spontaneous contractions of the DV tissue.

3 Experimental

3.1 Insect and Its DV Excision

The final stage larvae of the inchworm, Ctenoplusia agnata, were used in this study. The inchworms were raised continuously at 25 °C with only an artificial diet. Their DVs were excised under a stereomicroscope after surface sterilization in 70% ethanol solution. The excised DVs were cultured in the culture medium, TC-100 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution, at 25 °C.

3.2 Fabrication of the Frame

The frame for the PSMR was fabricated by molding PDMS (Figure 3). The mold was fabricated by machining a 1 mm thick poly(tetrafluoroethylene) (PTFE) sheet with a machining center (ROBODRILL, FANUC, Yamanashi, Japan). Then, uncured PDMS (Sylpod184, Dow Corning Toray, Tokyo, Japan) was poured onto the mold. Next, a slide glass was placed on the mold and they were pressed in a vise in order to remove excess uncured PDMS. After baking at 80 °C for 60 min, the frame with the slide glass was detached from the mold. Finally, to avoid tearing, the frame was carefully peeled off. In the case of the micropillar array, the PTFE mold was baked directly with excess uncured PDMS at 80 °C. After curing, the micropillar array was obtained by carefully peeling the PDMS film off.

3.3 Assembly of DV Tissue onto the Frame

The frame was hydrophilized using an oxygen plasma asher (PIB-10, Vacuum Device, Ibaragi, Japan) and was coated with Cell Tak (BD Biosciences, Franklin Lakes, NJ, USA). The excised DV was wrapped using tweezers onto the frame in the culture medium while being viewed under the stereomicroscope. The PSMR was then incubated at 25 °C without medium replacement.

3.4 Image Analysis for Evaluation of PSMR

The PSMR observations were made at 25 °C in all the experiments. Deformation distance of the frame and moving distance of the PSMR were observed with a digital

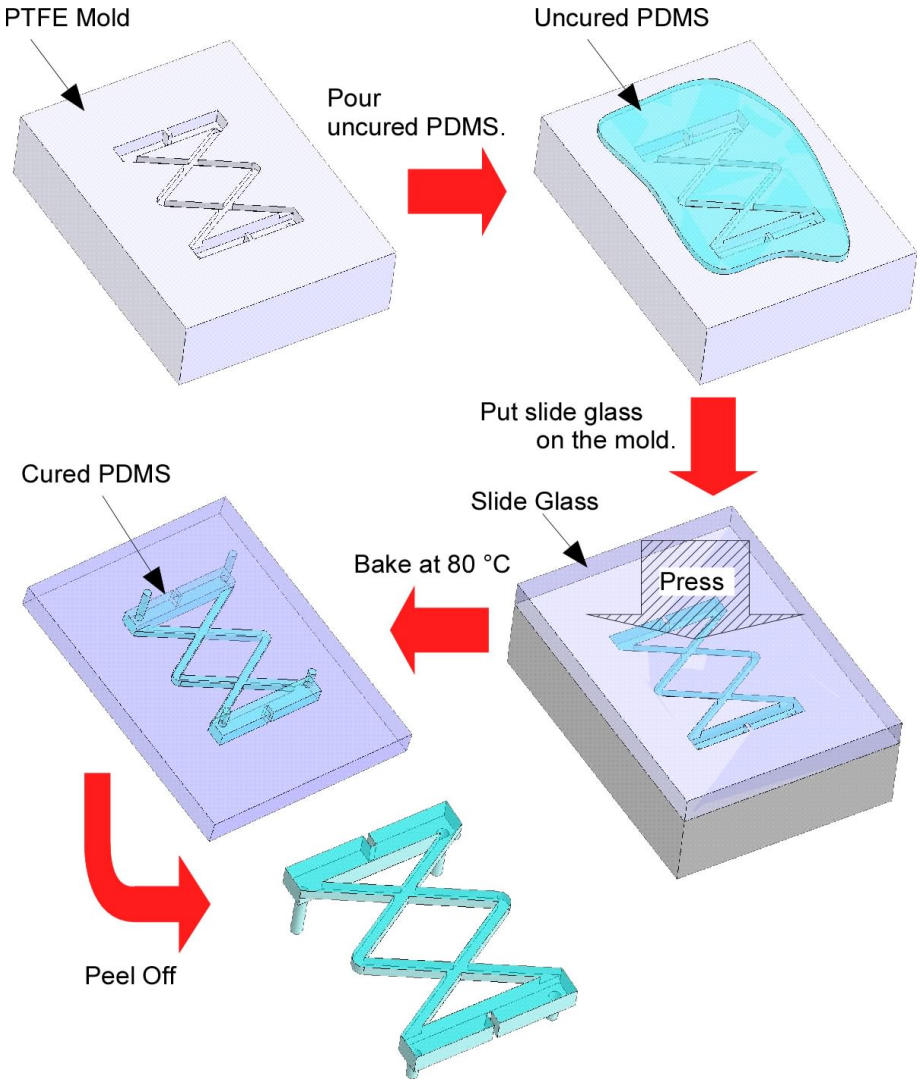

Fig. 3. Fabrication possesses for the PSMR frame made by PDMS molding

zoom microscope (KH-7700, Hirox, Tokyo, Japan) and a zoom microscope (AZ-100, Nikon, Tokyo, Japan) equipped with a CCD camera, respectively. The obtained microscopy movies were analyzed with analysis software (DippMotion, Ditect, Tokyo, Japan).

3.5 Evaluation of Insect Hormone

We tried to regulate the PSMR by adding insect hormone. We used the PSMR within a few days after assembling. CCAP purchased from LKT Laboratories (St. Paul, MN, USA)

was made up as stock solutions of 10^{-3} M using ultrapure water. The stock solution was stored at -20 °C. The concentration of CCAP in the culture medium was gradually increased by adding the stock solution or a diluted stock solution with TC-100 medium. CCAP was added to get a final concentration of 10^{-6} M. Before and after adding CCAP, the PSMR was observed as described in the image analysis above. The moving distance was measured with the DippMotion analysis software.

4 Results and Discussion

4.1 Movement Analysis of the PSMR

The deformation of the front and rear leg tips and moving distance of the PSMR was measured from a side view. Views of the relaxing and contracting PSMR are shown in Figure 4. Only part of the DV under the pantograph-shaped body contracted

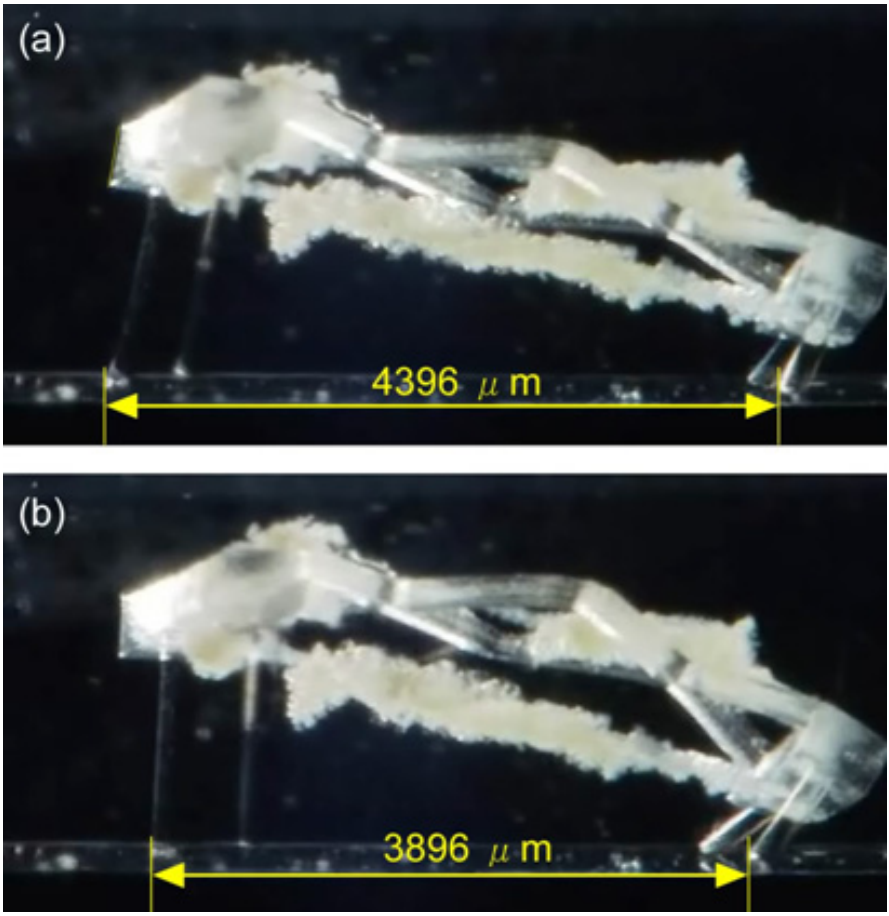

Fig. 4. Microscopic side views of the PSMR when relaxing and contracting

spontaneously, that action bowed the pantograph-shaped body. As a result, the distance between the front leg and the rear leg was reduced by 500 μm from 4396 μm to 3896 μm. The measured value was almost the same as the predicted value, 420 μm. We attribute the difference to the stiffness of the PDMS which depends on baking time and temperature and variability of the contractile force of DVs among individuals.

The movement of the PSMR was analyzed by image analysis (Figure 5). During 30 s, the PSMR moved 793 μm while the DV tissue contracted 12 times. Based on these results, the average stroke and velocity were calculated as 66.1 μm and 26.4 μm/s. The average stroke was much smaller than the reduced distance of 500 μm between the front and the rear legs. This shows the contractile force of the DV was utilized poorly. The efficiency could be improved by optimizing the shape of the leg tips. For instance, it is desirable that the shapes for front and rear legs allow the front legs to stick and the rear legs to slip when the DV contracts and conversely, the front legs to slip and the rear legs to stick when the DV relaxes. If the contractile force of the DV is evoked efficiently by improving the shape of the tip, theoretically, the velocity of the PSMR will increase to 200 μm/s.

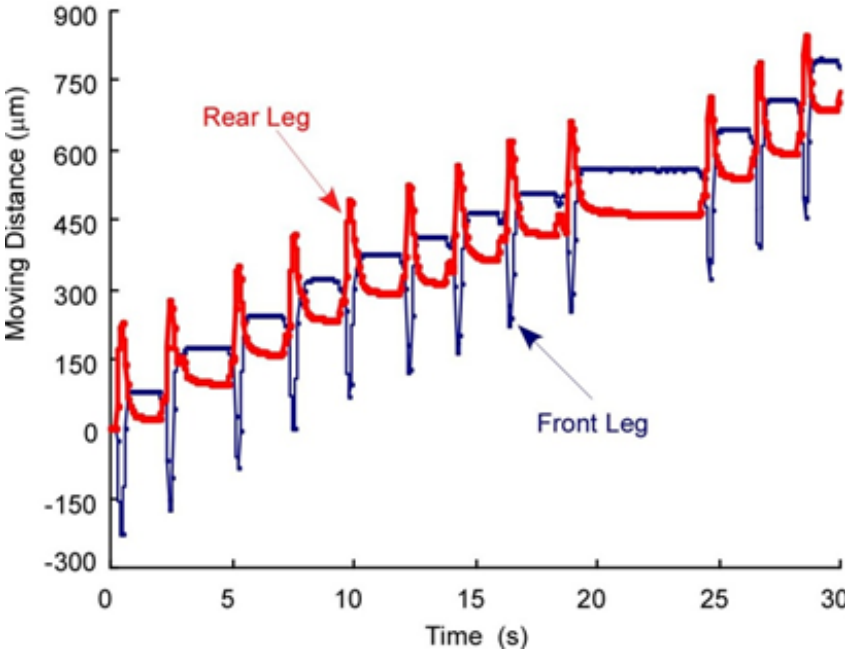

Fig. 5. Time course of positions of the front and rear leg tips. The positive direction of the Y axis was set to the designed moving direction from the starting position.

In general, the contractile force of muscle tissues depends on their own length and the maximum force is produced when they contract from the resting length. Therefore, it is important to wrap the DV onto the pantograph-shaped body with a

small tension because the DV shrinks less than its resting length as soon as it is excised from the insect. On the contrary, the contractile force of the DV decreases when the DV is extended excessively.

The PSMR was observed with a scanning electron microscope after fixation with paraformaldehyde. As shown in Figure 5, the DV under the pantograph-shaped body was wrapped tightly and the DV over the pantograph-shaped body was wrapped loosely. In these experiments the DVs were wrapped manually, but it is difficult to produce a large number of PSMRs with exactly the same wrapping conditions. Further research is needed to identify the relationship between length and contractile force of the DV and to develop a way to assemble the DV onto the frame with the desirable tension.

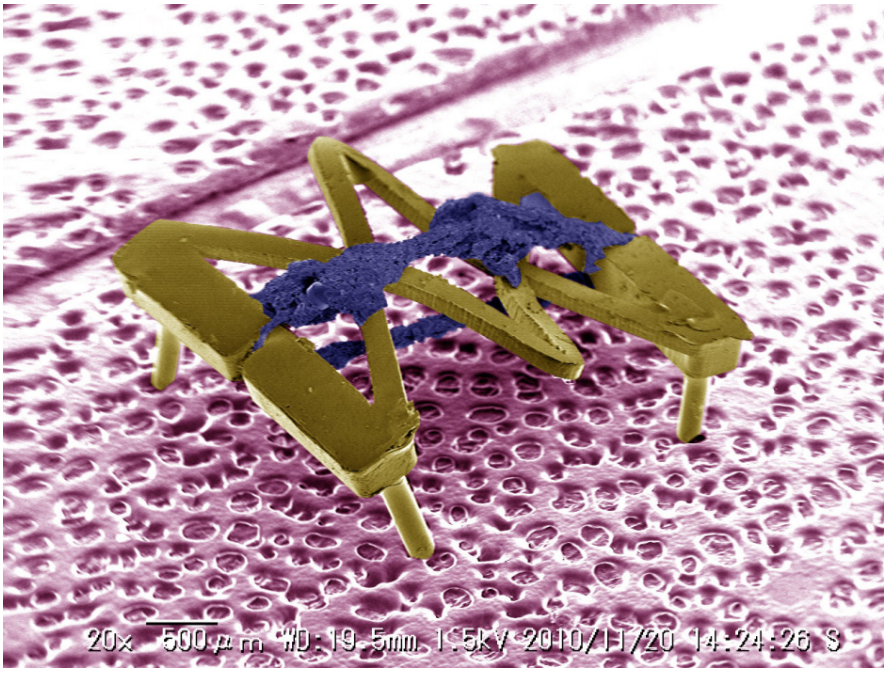

Fig. 6. Scanning electron microscope image of the PSMR

4.2 Acceleration by Adding CCAP

The trajectories of the PSMR before and after adding CCAP were analyzed and compared and the results for the first 30 s are show in Figure 7. The contractile frequency of the DV and the moving velocity of the PSMR were clearly increased by adding CCAP. The moving distances for 30 s before and after CCAP addition were 114 μm and 723 μm, respectively. The result indicates that the velocity of the PSMR increased 6.3-fold by adding CCAP at the final concentration of 10^{-6} M. We also calculated that the moving velocities before and after addition were 3.8μm/s and

24.1μm/s. The moving velocity before addition is much lower than that of the PSMR in the previous section. This is because the contractile frequency of the DV used in this experiment was lower than that of the DV used in the previous experiment and the friction force between the leg tips and the bottom of the culture dish differed between them.

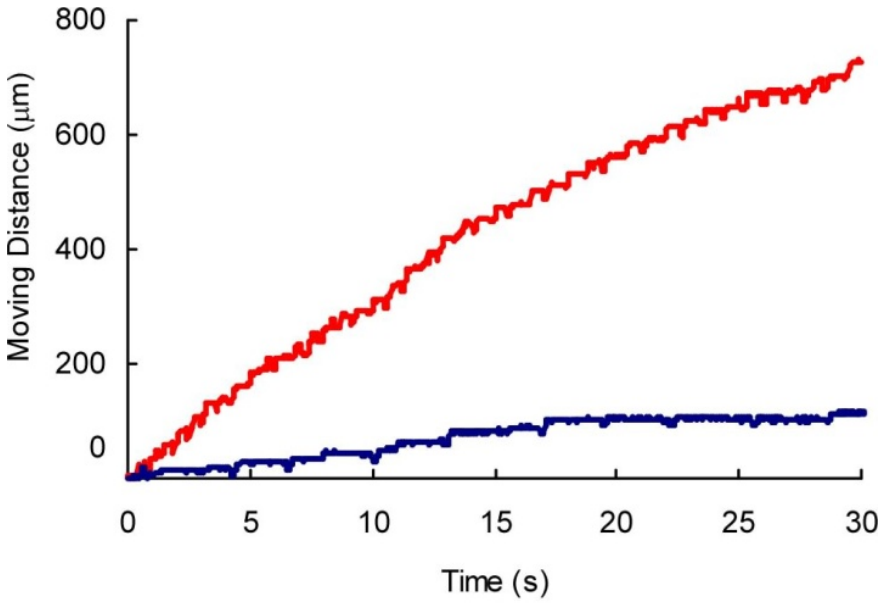

Fig. 7. Trajectories of the PSRM for 60 s before (blue line) and after (red line) CCAP addition. The frequencies before and after CCAP addition for 30 s were 0.43 Hz and 1.33 Hz, respectively.

5 Conclusion

We succeeded in fabricating the PSMR which autonomously moved at 25 °C. The PSMR was fabricated by assembling a whole DV onto the frame made of PDMS. The distance between the front and the rear legs was reduced by 500 μm when the DV contacted, which was almost equal to the value obtained by simulation. However, the moving distance in one contraction was 66.1 μm, which was much smaller than the reduced distance between the front and rear legs. The velocity obtained in the experiment was 26.4 μm/s. The velocity can be increased by improving the shape of the leg tips so as to make a difference in friction forces between the leg tips and the surface over which the body is traveling. We also confirmed that the PSMR could be accelerated temporally by adding CCAP, a kind of insect neural peptide. The velocity of the PSMR was increased 6.3-fold by adding CCAP. These results indicate that the DV is strong enough to be utilized as a microactuator which can be regulated by adding a chemical agent. Our group has also successfully light-regulated DV tissue

contraction of a fly by expressing channelrhodopsin-2, a directly light-gated cation-selective membrane channel found only in muscle tissues, and then irradiating the excised DV tissue with blue light [15]. In conclusion, the results in these experiments suggest that the insect DV has a higher potential for being a biological microactuator than other biological cell-based materials. Insect dorsal vessel (DV) tissue seems well suited for chemically regulatable microactuators due to its environmental robustness and low maintenance.

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