FABRICATION AND EVALUATION OF TEMPERATURE-TOLERANT BIOACTUATOR DRIVEN BY INSECT HEART CELLS

Y. Akiyama, K. Iwabuchi, Y. Furukawa, and K. Morishima
Tokyo University of Agriculture and Technology, Japan

ABSTRACT

This paper demonstrates micropillar actuation by insect heart cells over a wide range of temperature without medium replacement. We have fabricated a bioactuator using insect heart cells which are generally tolerant of temperature changes and have evaluated its temperature dependence. The bioactuator was able to work autonomously at 5 to 35 °C without irreversible damage, but it was irreversibly-damaged at 40 °C. The results suggest that the insect cell may have a high applicability for a temperature-tolerant bioactuator.

KEYWORDS: bioactuator , temperature-tolerant, insect, dorsal vessel

INTRODUCTION

Living muscle cells can be considered as a micro linear actuator with high efficiency which can work only with chemical energy. Several hybrid devices using mammalian cardiomyocytes were reported so far [1-3]. However, the mammalian cardiomyocytes can survive and contract spontaneously only under the optimal culture conditions at 37 °C and at pH of around 7.4. Due to their poor environmental robustness, the application of the device is restricted. On the other hand, an insect cell can generally survive and proliferate at 20 to 30 °C and at pH 6 to 8. We already proposed to utilize insect heart cells as an environmentally robust actuator [4] and reported a micropillar actuator driven by insect heart tissue [5]. The insect heart is called dorsal vessel DV, the shape of which is a long and thin tube. In this paper, A micropillar actuator driven by DV tissue is fabricated and temperature dependence of the actuator is evaluated by image analysis of displacement of a micropillar top.

EXPERIMENTAL

The micropillar actuator was fabricated as follows (Figure 1). An excised DV tissue excised from a caterpillar of *Ctenoplusia agnata* was plated on a PDMS micropillar array which was coated with Cell-Tak after oxygen plasma treatment. The micropillar array with DV tissue were soaked in TC-100 insect cell culture medium supplemented with 20 % fetal bovine serum and were incubated at 25 °C without medium replacement.

The DV-derived cells were migrated from the tissue and adhered to micropillars after a few days. After one week, the micropillars were actuated by DV cells and their displacements were over 20 μm (Figure 2). So as to assess the effect of temperature change, the actuation was observed at various temperatures from 5 to 40 °C. The displacements of micropillar tops were analyzed with image analysis software (ImageJ, NIH) after incubated at a targeted temperature for 1 hour.

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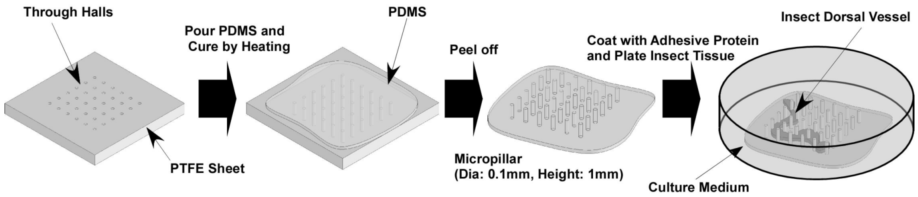

Figure 1. Fabrication process of the insect micropillar actuator.

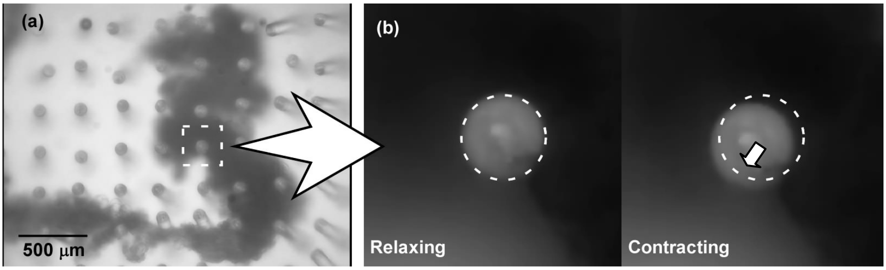

Figure 2. Microscopy images of micropillars with dorsal vessel tissue (a) and enlarged images of the micropillar relaxing and contracting (b)

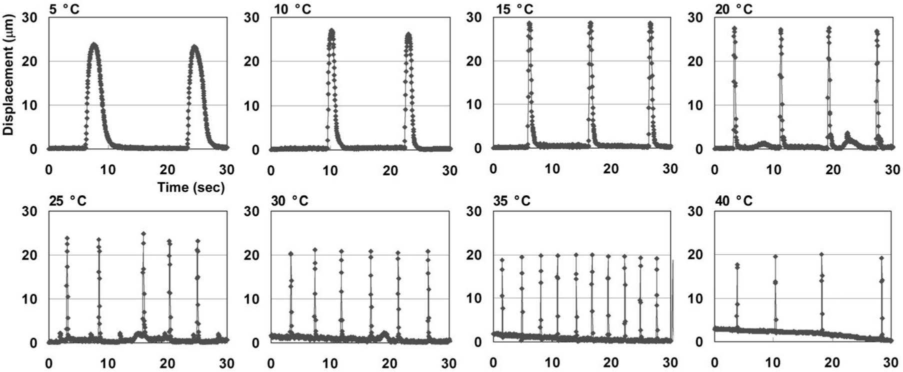

Figure 3. Image analysis results of micropillar displacements at each temperature.

RESULTS AND DISCUSSION

The dorsal vessel kept contracting spontaneously at every temperature between 5 to 40 °C (Figure 3). Based on the image analysis results for one minute, the contractile rate, frequency, and displacement were calculated and shown in Figure 4. Though the exposure to 5 °C for one hour weakened the contraction temporarily, the contraction was recovered by warming the medium to 25 °C. On the other hand, the exposure to 40 °C for one hour caused irreversible damage to the DV cells and the contraction was not recovered by cooling it 25 °C.

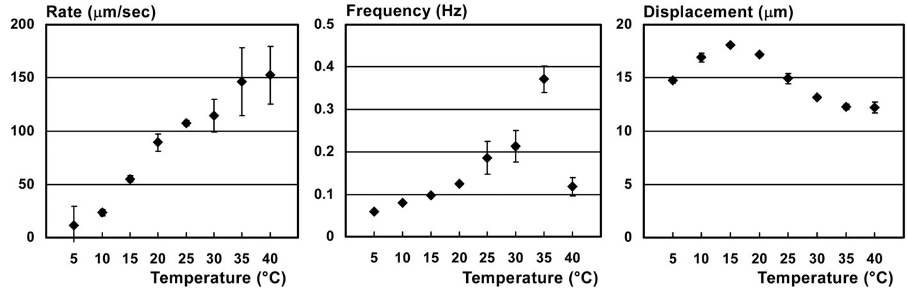

Figure 4. Contraction frequency, oscillation, and rate as a function of temperature.

The contraction rate increased with rising temperature at 5 to 40 °C. The contraction frequency increased as temperature rose until 35 °C, but the frequency dropped at 40 °C owing to irreversible thermal damage. The contraction oscillation peaked at 15 °C and decreased after that temperature. This data is correspond with the data of other animal's muscle. According to material mechanics, the displacement is proportional to the contractile force of the DV cells since this micropillar can be considered as a cantilever beam.

The relationships between temperature and contractile rate, frequency, and force were manifested. These graphs show the DV cell is not only temperature-tolerant compared with mammalian muscle cells but also possibly to regulate spontaneous contractions by temperature change.

CONCLUSIONS

The temperature dependence of caterpillar DV cells was evaluated so as to create a temperature-tolerant bioactuator. Though a cardiomyocyte can contract spontaneously only around 37 °C, the DV cells could contract spontaneously at 5 to 35 °C without irreversible damage. Furthermore, we already reported the insect cell can contract spontaneously over 90 days without medium replacement. Hence, the insect cell is much more robust over culture conditions than rat cardiomyocytes using which the microfluidic devices were already prototyped. Taking these merits into account, the insect DV cell seems to possess a high potential as a bioactuator element.

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