BIO-HYBRID CAPILLARY PULSATION DRIVEN BY A HEART MUSCLE OF INSECT

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ABSTRACT

A micropump driven by a heart muscle of insect was designed and demonstrated in this work. To realize bioactuator with self-contraction and environmentally robustness in a microfluidic device, an insect muscle tissue as a power source was applied for *in-situ* liquid flow generation. A diaphragm-based micropump was designed, fabricated and assembled with an insect muscle tissue. The micropump generated pulsation flows in a microchannel at room temperature without CO_2 control for the tissue culture. The insect tissue based bioactuator with robustness will be widely useful for in-vitro models of circulatory organ and tissue.

KEYWORDS: Bioactuator, Micropump, Muscle Contraction.

INTRODUCTION

Pulsation flows in dorsal blood vessels are essential for physiological micro-environments of tissue organs. Although many micropump devices for in-vitro model with outer/inner actuators have been developed, precise controls and connections with electrical/mechanical systems to generate biological flows are required [1]. Recently, engineered muscle tissue or cell sheet made from mammalian cells were proposed as bioactuators [2-4]. In this paper, a bio-hybrid micropump driven by a heart muscle of insect (larva), which is expelled from agricultural fields, is proposed. Insect muscle can be used as a robust bioactuator at room temperature for a long term [5-8]. With the integration of a muscle tissue in a microfluidic chip, in situ and feeble pulsation in a microchannel without any complicated connections was generated similarly to biological capillary blood flows.

Figure 1. A Larva (Ctenoplusia agnate) as biomaterial for bioactuator.

DESIGN AND FABRICATION

A micropump system consists of the microchannel and the diaphragm membrane with the base for a muscle tissue clamp over the microchannel as shown Figure 2. When the clamped muscle tissue contracts, the base leans and lifts the diaphragm membrane. And then liquid in the microchannel introduces into the diaphragm chamber (Figure 2B). Following the relaxation of the muscle, the diaphragm membrane becomes even and pushes liquid out (Figure 2A). As the heart muscle contracts spontaneously, the pulsation flows are formed in the microchannels. To realize the pump mechanism with robustness at the room temperature, a insect heart muscle tissue (length: ~ 4 mm, width: ~ 50 μm), which is isolated from a larva of *Ctenoplusia agnate* (Figure 1) and has the advantages of the contraction force (~ 90 μN) with the robustness of the wide range conditions of temperature and medium pH [5-8], was used as an engine for the micropump. According to the contraction force and the size of the muscle tissue, the micro-scale diaphragm membrane (diameter: 1.0 mm, thickness: 10 μm) and the microchannel structures were confirmed to generate the sufficient displacement (~ 100 μm) of the diaphragm using the finite element method based simulation

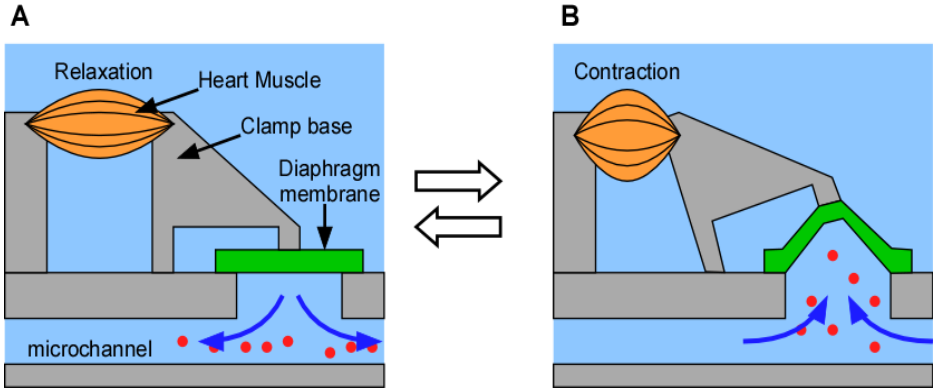

Figure 2. Cross-sectional schematic diagrams of the micropump mechanism driven by contractions of a muscle tissue. The structures are (A) A clamped muscle tissue relaxes in medium. (B) A muscle tissue contracts and then the diaphragm membrane is lifted.

software (COMSOL® multiphysics) as shown Figure 3. The two clamp bases and microchannel made of polydimethylsiloxane (PDMS) were fabricated using conventional photolithography technique with SU-8® (MicroChem) and PDMS molding process. Briefly, a precise Cr-photomask for the clamp bases, diaphragm membrane, and microchannel was fabricated using a maskless lithography system (DL-1000; NanoSystem Solutions, Tokyo, Japan). The photomask was then exposed to UV light using a mask aligner to produce the structure of the SU-8 50 photoresist (MicroChem, Newton, MA, USA) on a silicon wafer. The PDMS parts and microchannel were formed by replicating the SU-8 master using PDMS prepolymer (Sylgard 184 silicone elastomer kit; Dow Corning, Midland, MI, USA). After the inlet and outlet holes were punched, the PDMS chip was bonded to a glass slide by O_2 plasma treatment. The depth and width of the microchannels were 50 μm and 100 μm respectively.

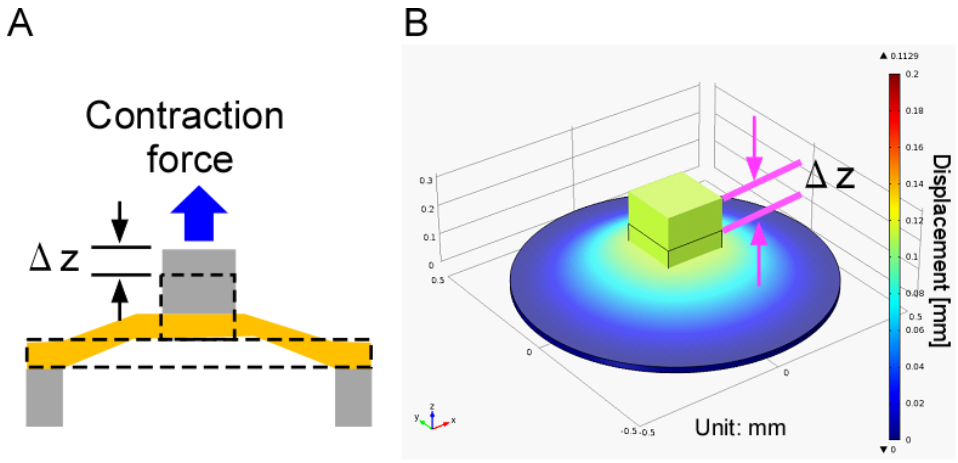

Figure 3. An estimation of the displacement of the diaphragm membrane. (A) A schematic of the cross-sectional structure of the diaphragm membrane pulled by contraction force. The shape of dot line shows the membrane attached the base before the muscle contraction. Δz means the z-axis displacement of the top of the membrane. (B) A computational simulation (COMSOL Multiphysics) of the deformation of the membrane with the force (90 μN) from an insect muscle. The PDMS membrane with 20 μm in thick and 1.0 mm in diameter is simulated.

ASSEMBLY OF A MUSCLE TISSUE

The microchannel chip with the diaphragm was immersed in insect culture medium and rotated with centrifuge at the rate of 3000 rpm to be filled with liquid containing fluorescent microbeads for the observation of pulsation flows in the microchannel. A heart muscle tissue was isolated from the larva and cultured in insect culture medium, TC-100 (Sigma-Aldrich) supplemented with 10% fetal bovine serum, at 25 °C for 6 hours before assembling the tissue. The heart muscle tissue was bridged between the two clamp bases (Figure 4B).

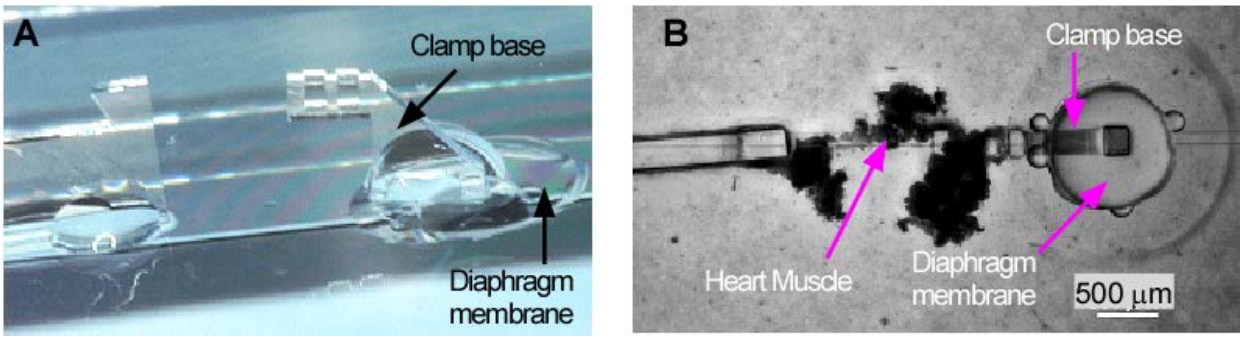

Figure 4. Microscopic images of the clamp base of muscle tissue on the diaphragm membrane. (A) The base without a muscle tissue. (B) A heart muscle tissue of insect clamped with the base.

RESULTS AND DISCUSSION

The simulation result with COMSOL shows that the contraction force causes 100 μm in displacement of the diaphragm membrane when an insect muscle tissue generates contraction force at 90 μN on the diaphragm PDMS membrane with the 20 μm in thick and 1.0 mm in diameter in the case of ignoring the liquid component (Figure 3B). Considering this result, we estimated that one contraction of the muscle tissue generates liquid flow of 0.16 μL at the maximum in the microchannel. In contrast, it is confirmed that the displacement of the diaphragm membrane was 4 μm (Δz in Figure 3A) from experimental observation of the motions of the clamp base with the muscle tissue. This experimental result indicates the generation of liquid pulsation of 6.3 nL per one muscle contraction. In addition, from the microscopic observation of microbeads in the pulsation flows as shown in Figure 6A, it was confirmed that the contraction of the assembled muscle tissue generated pulsation flows with the displacements of approximately 20 μm (Figure 5B).

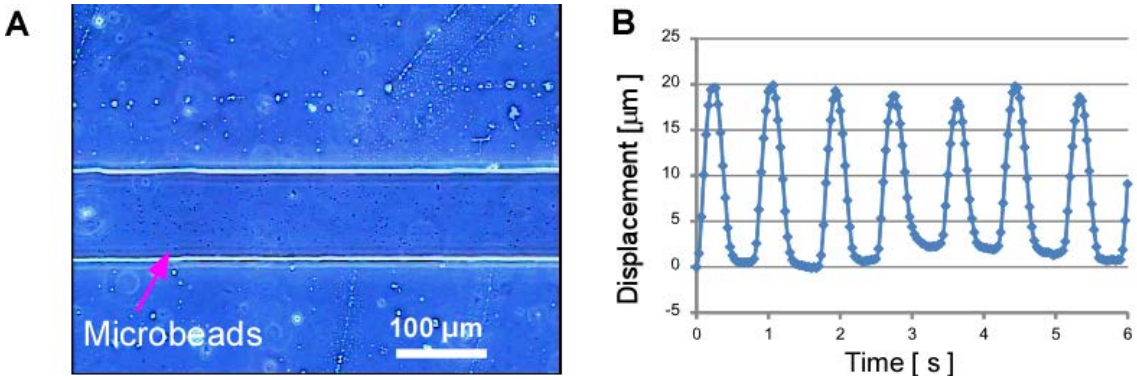

Figure 5. Measurement of pulsation flows driven by the muscle tissue in the microchannel. (A) A microscopic image of flows containing microbeads for the visualization of liquid flow. (B) The displacements of the microbead in the pulsation flows in the microchannel.

CONCLUSION

The micropump assembled with insect muscle tissue as an actuator for liquid pumping in a microchannel was proposed in this report. It was achieved that capillary pulsation was generated by insect muscle tissue contraction at room temperature although the experimental volume of pulsation flow generated by the muscle tissue was lower than that estimated by computational simulation. This bio-hybrid microfluidic device with temperature robustness has a potential applications not only for in vitro models of biosystems, but also for actuators as a transducer between chemical and mechanical energy in microdevices.

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