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Culture of insect cells contracting spontaneously; research moving toward an environmentally robust hybrid robotic system

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Abstract

Here we propose an environmentally robust hybrid (biotic–abiotic) robotic system that uses insect heart cells. Our group has already presented a hybrid actuator using rat heart muscle cells, but it is difficult to keep rat heart muscle cells contracting spontaneously without maintaining the culture conditions carefully. Insect cells, by contrast, are robust over a range of culture conditions (temperature, osmotic pressure and pH) compared to mammalian cells. Therefore, a hybrid robotic system using not mammalian cells but insect cells can be driven without precise environmental control. As a first step toward the realization of this robotic system, the larvae of two lepidopteran species, *Bombyx mori* (BM) and *Thysanoplusia intermixta* (TI) were excised and the culture conditions of their dorsal vessel (insect heart) cells were examined. As a result, spontaneously contracting TI cells derived from the dorsal vessel were obtained. The contraction of TI cells started on the 7th day and continued for more than 18 days. Spontaneously contracting BM cells were not obtained in this study. These experimental results suggest the possibility of constructing an environmentally robust hybrid robotic system with living cells in the near future.

Keywords: Hybrid robotic system; Insect cell; Spontaneous contraction; Dorsal vessel; Environmentally robust

1. Introduction

In recent years, there have been many studies on downsizing and integration of not only semiconductor devices but also mechanical systems and chemical systems, such as micro electro mechanical systems (MEMS) and micro total analytical systems (μTAS). Those systems and devices are driven by conventional technology i.e. a magnetic field, electricity and air pressure. The strategy to miniaturize such conventional systems as these is called a "top-down approach." However, the driving systems and the sensors in the micro systems to be treated by the top-down approach generally need a large-scale external system and have many problems regarding energy conversion efficiency and sensitivity.

Bio-molecular motors as an alternative driving source for micro devices have attracted much interest recently (Soong et al., 2000; Liu et al., 2002) because they can transduce chemical energy into mechanical energy efficiently without converting it into thermal energy, but the power generated by one molecule is several pN s^{-1} which is too small to drive a micro device. The strategy to build up nano devices is called a "bottom-up approach." But it is difficult to assemble bio-molecular motors in the intended position. Therefore, we focused on using muscle cells as a new initiative that falls between micro machining and bio-molecular motors in terms of both generated force and size (Fig. 1).

A few studies on the hybrid (biotic–abiotic) robotic system have been published. Reported examples of a robotic system using a biological component of a living body are a mobile robot mounting antennae of a silkworm moth as a very sensitive gas sensor (Kanzaki et al., 1994; Kuwana and Shimoyama, 1998) and a swimming robot actuated by explanted frog semitendinousus muscles (Herr and Dennis, 2004). Reported examples of

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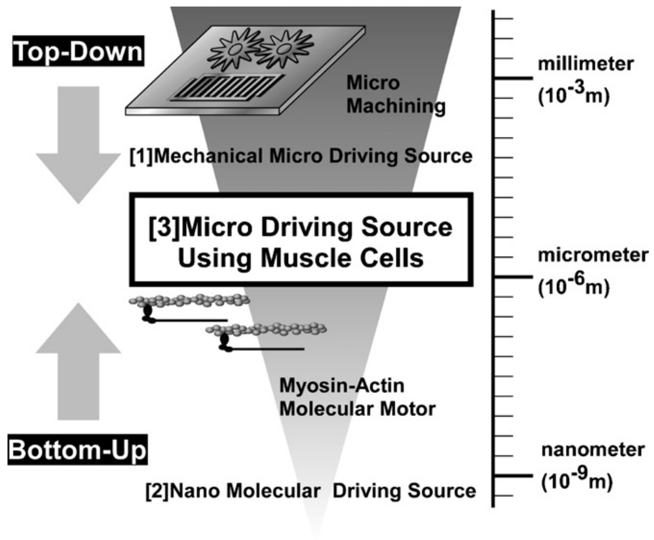

Fig. 1. Classification of microactuators. The majority of microactuators are fabricated by micro machining and actuated by mechanical and electrical energy. While bio-molecular motors, such as myosin–actin and bacterial flagellar motors, have attracted much interest recently, here we propose to use muscle cells as an alternative high efficiency transducer.

a micro robotic system using living cells include self-assembled silicon microdevices actuated by heart muscle cells (Xi et al., 2005) and a three-dimensional culture of muscle cells for powering biohybrid microdevices (Kim et al., 2006). However, no reports have been made on any study to build up a mechanical component with living cells. Our group, however, has reported on a micro bio-actuator using spontaneous beating of primary neonatal rat heart muscle cells (Morishima et al., 2006; Tanaka et al., 2006a,b) and a controllable actuator using skeletal muscle cell contraction evoked by electrical stimuli (Akiyama et al., 2006). These systems need the precise control of temperature and pH in medium in order to keep heart muscle cells contracting.

To construct a hybrid robotic component with living cells, tissue engineering (Langer and Vacanti, 1993) is the technology with the most potential. In muscle tissue engineering, various biomaterials, e.g. collagen (Okano and Matsuda, 1997), polyglycolic acid (Saxena et al., 2001), polyurethanes (Mulder et al., 1995), and polypropylene (Neumann et al., 2003), are currently used as biocompatible or biodegradable scaffolds. Engineered three-dimensional heart tissue which can beat spontaneously was reported that was obtained by gelling a mixture of cardiomyocytes and collagen solution (Eschenhagen et al., 1997; Zimmermann et al., 2002). Additionally, cell sheet engineering in which cells cultured on surfaces coated with temperature-responsive polymer can be harvested as a viable contiguous cell sheet simply by the lowering temperature without any enzymatic digestions was proposed (Yamada et al., 1990) and was applied to myocardial tissue reconstruction (Shimizu et al., 2003). Such techniques enable researchers to construct three-dimensional muscle tissues.

However, there are a lot of issues to be resolved. In particular, the internal cells of the tissue often die when the engineered tissue is thicker than a few millimeters. The current tissue engineering cannot construct a complete tissue from cells by itself. The one big reason for this failure is that the current technology cannot construct blood capillaries within the tissue. Therefore, the internal cells of the tissue cannot metabolize and they necrotize. Neoangiogenesis is one of the most important subjects to be dealt with in constructing muscle tissues *in vitro*.

Insects which are the most abundant animals on earth can be regarded as a completed mechanical system. They can survive under very poor living conditions compared with more highly evolved animals like mammals, birds and amphibians. Unlike mammal cells, in general insect cells do not need a precise control since they can tolerate a range of culture conditions, including temperature, osmotic pressure and pH. For instance, the cells derived from *Ephedrus plagiator* can proliferate at 10 °C (Eppler et al., 1980), the cells derived from *Heliothis zea* ovary can proliferate at pH between 6 to 8 (Kurtti and Brooks, 1972) and the cells derived from *Heliothis zea* ovary and from *Drosophila melanogaster* embryo can survive at osmotic pressures between 230 and 380 mOsm kg^{-1} (Kurtti et al., 1975) and 225–400 mOsm kg^{-1} (Wyss and Bachmann, 1976), respectively. Furthermore, insects do not have capillary blood vessels; alternatively they have an open vessel system (Fig. 2). So neoangionenesis is unnecessary in tissue construction with insect cells, which indicates it may be possible to construct tissues and organs simply. These points are significant for utilizing cells as a robotic system.

In this study, we propose a hybrid (biotic–abiotic) robotic system that is tolerant to different environments. We use an alternative approach to construct a biotic robotic component with insect living cells. As a first step, to obtain the cells which can contract spontaneously at room temperature, we excise and culture dorsal vessels of lepidopteran larvae. This strategy will provide a base for constructing a biotic component for eventual incorporation into a hybrid robotic system.

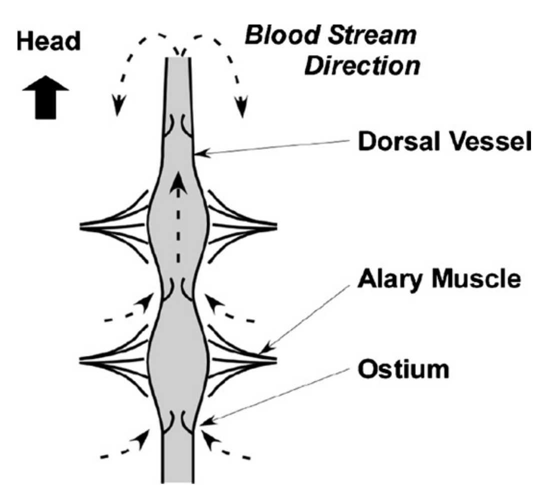

Fig. 2. Heart anatomy of lepidopteran larva. The insect has an open vessel system i.e. it does not have any blood capillaries. Therefore, the blood pumped by the dorsal vessel returns there through ostia after flowing through the intercellular spaces all over the insect's body. The alary muscle assists in sending blood by expanding the dorsal vessel.